# National Institute of Child Health and Human Development (U.S.)

# Annual Report of Intramural Research

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October 1, 1989
to
September 30, 1990

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01500-08 OSD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies on DNA Replication, Repair, and Mutagenesis in Eukaryotic and Prokaryotic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A.S. Levine, Head, Section on Viruses and Cellular Biology, OSD, NICHD

| | | | | |
|---|---|---|---|---|
| Other: | C.T. Patch | Sr. Investigator | C. Ho | Visiting Fellow |
| | M. Protic | Visiting Scientist | E. Chumakov | Visiting Fellow |
| | R. Woodgate | Sr. Staff Fellow | J. K. Carr | NRC Biotech. Fellow |
| | M.P. Carty | Visiting Assoc. | F. Pompetti | Guest Researcher |
| | M. Carbone | Visiting Assoc. | M. Zernick | Guest Researcher |
| | C. Cicala | Visiting Fellow | J. M. Hauser | Microbiologist |
| | M. Abramic | Visiting Fellow | M. McLenigan | Chemist |

COOPERATING UNITS *(if any)*

LDMI, NICHD ( S. Hirschfeld & K. Ozato); SGF (OSD), NICHD (M. Oshima); GBB, NIDDK (E. Ackerman); LMC, NCI (K. Kraemer); U. of Cincinnati (K. Dixon) ; FDA (W. Koch); Office of Naval Research (E. Eisenstadt); LMG, NICHD (D. Ennis).

LAB/BRANCH

Office of the Scientific Director

SECTION

Section on Viruses and Cellular Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 13.5 | PROFESSIONAL: 11.5 | OTHER: 2.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In eukaryotic cells, the SV40-based shuttle vector, pZ189, has been utilized *in vivo* and *in vitro*, to analyze mutations in response to UV and benzpyrene (BP). These two agents yield different frequencies and specificities of mutations *in vivo*. Moreover, mutation fixation (with similar spectra as *in vivo*) occurs during replication of UV and BP-damaged pZ189 *in vitro* (using cell extracts plus SV40 large T antigen to promote DNA replication). In a gap-filling mutagenesis assay, purified polymerase α (HeLa cell) is highly inaccurate during DNA synthesis, but accuracy is improved several-fold if HeLa single-stranded DNA binding protein (SSB) is also present. Two BP enantiomers, (+) and (-)anti, form adducts with DNA, but the (+) form is far more carcinogenic. When pZ189 was adducted with the two enantiomers, and the vector "shuttled" *in vivo*, the (+) form had a greater effect on the replication complex both in terms of lesion bypass and nucleotide misincorporation. In the *in vitro* replication system, the (+) form again had a greater effect. Studies in *E.coli* have focused on the UmuD cleavage reaction, which is inefficient *in vitro* but is required for mutagenesis *in vivo*. We now find, using an ultrasensitive chemiluminescent assay, that UmuD cleavage is also inefficient *in vivo*. To elucidate the role of the vital but inefficiently produced cleavage product, UmuD', we have over-produced UmuD' protein by placing an engineered UmuD' gene under the control of the strong lambda $P_L$ promoter. Unlike *E.coli*, *S.typhimurium* is poorly mutable. However, we found that the Salmonella UmuD protein is cleaved to UmuD', indicating that the defect in mutagenesis is not due to a defect in processing. When mammalian cells are pretreated with a carcinogen (UV or mitomycin C), the repair of UV-damaged DNA (in the form of a transfected vector) is enhanced. We have identified a constitutive DNA damage-specific, DNA binding (DDB) protein complex which is induced to high levels by this pretreatment. DDB protein is the first such damage-specific, damage-inducible DNA binding protein identified in primate cells. DDB protein is not detectable nor inducible in patients with the repair deficient, cancer-prone disease, xeroderma pigmentosum. Thus, DDB may play a central role in DNA repair. With regard to DNA replication, our focus is on a viral gene product, SV40 small t antigen (tag). This protein is required for the efficient induction of cellular DNA replication and transformation when non-dividing cells are infected *in vitro* by SV40, while large Tag alone suffices in proliferating cells. We found that SV40 tag deletion mutants readily transform rapidly proliferating cell types (e.g., lymphoblast, osteoblast) *in vivo*, leading to lymphomas and osteosarcomas in infected hamsters; animals infected with wild type SV40 develop tumors of nonproliferating cells (i.e., fibrosarcomas). SV40 tag may effect a change in the phosphorylation state of Tag, which may in turn be necessary for Tag-induced DNA replication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00093-16 OSD

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Action of Nerve Growth Factor

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | G. Guroff | Head | OSD, NICHD |
| Others: | See Attached | | |

COOPERATING UNITS (if any)

See Attached

LAB/BRANCH

Office of the Scientific Director

SECTION

Section on Growth Factors

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland, 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 8.50 | PROFESSIONAL: 7.25 | OTHER: 1.25 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Nerve growth factor (NGF) is a polypeptide required for the survival, development, and differentiation of sympathetic and sensory neurons. It is also trophic for certain neurons in the central nervous system and has effects on a number of tumor cell lines. NGF binds to specific receptors on the surface of its target cells, initiates a chain of intracellular events, and, through these intracellular actions, controls the expression of specific genes. It is the summation of these intracellular actions and gene expressions that constitute the developmental instructions NGF imparts to responsive cells. The molecular mechanism(s) by which the factor controls gene expression is not known in fine detail, but, through the work in this and other laboratories, the cascade of intracellular events initiated at the receptor is becoming clarified. Substantial progress has also been made toward an understanding of which genes are required for NGF action and how through the study of the very informative cell line, PC12. When treated with nerve growth factor, PC12 cells stop dividing, elaborate neurites, become electrically excitable, and will synapse with appropriate muscle cells in culture. PC12 has become a premiere tool for the study of nerve growth factor, and has received substantial attention as a model for neuronal differentiation in general. Using the PC12 system, we and others have observed the NGF-induced increase of a number of second messengers. These changes in second messenger levels could be expected to alter the phosphorylation patterns in the cell and, accordingly, we and others have observed a number of such changes. These include increases in the phosphorylation of a nuclear protein called SMP and the ribosomal protein S6, and a decrease in the phosphorylation of the soluble protein, elongation factor 2 (EF-2). Biochemical dissection of these changes in phosphorylation has led to the concept that NGF acts in the cell by activating a number of parallel phosphorylative cascades, all emanating from the receptor, but keyed by different second messenger systems. These cascades carry the NGF signal to various parts of the cell, including the nucleus, where the changes in the phosphorylation of key proteins cause changes in their function. The changes occurring in the nucleus could, then, lead to changes in the expression of specific genes. As yet unknown is the chemistry of the reaction occurring immediately after the combination of NGF with its receptor and linking this signal with the relevant kinases in the cell. It has been suggested that this coupling is accomplished by the combination of the receptor with specific proteins recruited from the soluble portion of the cell. It is reasonable to hope that an understanding of the actions of nerve growth factor will shed light on the process of neuronal differentiation.

| | | | |
|---|---|---|---|
| Others: | G. Dickens | Biological Laboratory Technician | OSD, NICHD |
| | B. Nikodijevic | Visiting Scientist | OSD, NICHD |
| | N. Kaneda | Visiting Scientist | OSD, NICHD |
| | Y. Hirata | Visiting Associate | OSD, NICHD |
| | D. Fink | Staff Fellow | OSD, NICHD |
| | M. Tocco | Visiting Fellow | OSD, NICHD |
| | M. Oshima | Visiting Fellow | OSD, NICHD |
| | M. Mogi | Visiting Fellow | OSD, NICHD |
| | M. Sutphin | Federal Junior Fellow | OSD, NICHD |

Cooperating Units:

Laboratory of Biochemistry, National Heart, Lung, and Blood Institute, NIH (S. Rhee)
Laboratory of Viral Carcinogenesis, National Cancer Institute, NIH (U. Rapp)
Department of Pathology, Washington University School of Medicine (J. Milbrandt)
School of Pharmacy, The Hebrew University of Jerusalem, Israel (P. Lazarovici)
Department of Internal Medicine, Fukui University, Japan (T. Mutoh)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00054-16 LCE

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Behavior Analysis of Vocal Communication in Squirrel Monkeys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | D. Symmes<br>(After 5/90: M. Biben) | Head, BBCS | LCE, NICHD |
| Other: | M. Biben | Expert | LCE, NICHD |
| | D. Bernhards | Bio. Lab. Tech | LCE, NICHD |

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Brain, Behavior, and Communication

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 1.4 | 0.7 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

SBBC continues to focus on the vocal behavior of nonhuman primates, using the squirrel monkey as a model for basic and universal truisms of language. Ape and monkey vocal communication has traditionally been seen as highly programmed genetically and closed to learning, in contrast to the acquisition of language skills by human infants or even by birds. Early work in European labs indicated that, for the squirrel monkey, vocal production is at birth equivalent to that of adults. However, our data recently collected from 6 infant subjects over the first year of life, indicate that developmental changes clearly do occur, although certainly not of the magnitude of human language acquisition. Behavioral data collected along with the production data have revealed that it is aunts (female relatives or unrelated friends of the mother) and not the mothers themselves who interact vocally most often with very young infants, and that it is aunts who elicit the first vocal responses.

Among adult squirrel monkeys, the exchange of chuck vocalizations has been shown by this laboratory to have many of the characteristics of human conversation. Using a technique of recording animals' voices and then playing them back, this past year we showed that females responded significantly more often to the chucks of group members than to those of strangers. We are currently testing the ability of females to discriminate a very sophisticated (for primates) and acoustically subtle (for humans) distinction between chucks uttered either first or second in a dialogue, a sequence which has informational properties comparable to a question and answer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00062-14 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Physiological Correlates of the Infant Cry and Related Vocalizations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | J. D. Newman | Head, UN, CBGS | LCE, NICHD |
| Other: | J. L. Norcross | Pre-IRTA Fellow | LCE, NICHD |
| | L. J. Crepeau | IRTA Fellow | LCE, NICHD |

COOPERATING UNITS *(if any)* LCS, NIMH (Murphy); LN, NIMH (Bachevalier); Div of Child Psychiatry, Johns Hopkins Sch of Med (Harris); Dept. of Pediatrics, Johns Hopkins Sch of Med (DeAngelis); Dept. of Zoology, U of Md (Carter, Tamarkin); BSB, NIMH (Huffman); Purdue U (Bryan).

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 2.0 | .0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies examine the neuroanatomical and neuropharmacological mechanisms related to production of the isolation call and related vocalizations in nonhuman primates, as a model for understanding the neurology and physiology of the vocal expression of affect in human infants. Major findings this year were as follows: 1) Attempts to confirm in a primate model a role for the neuropeptide cholecystokinin in mediating panic attacks were unsuccessful. However, during the course of these experiments we discovered a clear difference in the reactivity profile of male and female squirrel monkeys to the mildly stressful contexts of brief social separation and exposure to alarm-evoking visual stimuli. Males show greater locomotor activity than females during both test situations, and more vocal activity during separation. Females vocalize more during alarm-provoking testing. Furthermore, males and females exhibit different responsivity to administration of the anticholinergic drug benactyzine, in that males decrease locomotor activity and increase alarm-provoked vocal activity to levels of untreated females, whereas females show no change in locomotor activity but do increase alarm-provoked vocal activity to levels 2-3 times above vehicle trials. 2) the role of the amygdala and inferotemporal cortex in regulating male and female vocal responses to brief periods of social separation was clarified in infant rhesus macaques. The tendency for male infants to produce more tonal and less variable vocalizations in this context disappears following bilateral ablation of the inferotemporal cortex. Conversely, bilateral ablations of the amygdala, which receives input from IT cortex, result in a more severe acoustic alteration in the separation calls of female infants. 3) analysis of the cries of premature infants with intraventricular hemorrhage (IVH) indicates a hyporesponsiveness to routine manipulations, manifested by weak, poorly elaborated cry patterns. Following these infants after discharge from the intensive care nursery will permit assessment of cry acoustic pattern changes in association with resolution of the IVH diagnosis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 00702-10 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Genetics of Primate Vocal Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J. D. Newman | Head, UN, CBGS | LCE, NICHD |
| Others: | J. L. Norcross | Pre-IRTA Fellow | LCE, NICHD |

COOPERATING UNITS *(if any)* Dept. of Zoology, U of Maryland (C.S. Carter, L. Tamarkin); Marmoset Progect, ORAU (S. Tardiff, N. Clapp), National Zoological Park (W. Xanten); Purdue University (Y. E. Bryan).

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | .0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Considerable progress was made this year in implementing the operation of a new signal processing workstation, permitting more rapid fine-grained analyses of the acoustic structure of a variety of vocalizations, as well as producing artificial sound sequences used in playback experiments. One focus this year was to improve our understanding of how perception of the infant cry and marmoset phee call affects the behavior of listeners. Playback studies using natural and synthetic vocal sequences were conducted, using vocal 'answers' as a measure of interest in the marmoset study, and heart rate change as a measure of interest in the infant cry study using adult human listeners. Additionally, considerable progress was made in developing an assay for LH from urine and blood samples collected from our breeding colony of marmosets. This assay will permit identification of ovulatory cycles in specific individuals, useful both for assessment of breeding success and to correlate with cyclic changes in vocal activity. Significant gender differences were found in the association between reactivity and cry acoustics in human infants, and in the acoustic properties of species-specific territorial vocalizations in adult common marmosets.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01106-07 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Developmental Continuity of Individual Differences in Rhesus Monkey Reactivity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I. | S. J. Suomi | Head | LCE, NICHD |
| Others: | K. L. Rasmussen | Senior Staff Fellow | LCE, NICHD |
| | M. Champoux | Staff Fellow | LCE, NICHD |
| | K. Abbott | Research Psychologist | LCE, NICHD |
| | J. D. Higley | Staff Fellow | LCS, NIAAA |
| | K. A. Bard | NRSA Fellow (Individual) | Yerkes Primate Center, Emory University |

COOPERATING UNITS *(if any)* DEB, NICHD (Wheler, Loriaux); LCS, NIAAA (Linnoila); Delta Reg Primate Ctr. (Clarke); Yerkes Reg. Primate Ctr. (Nadler); Primate Lab, U of Wisconsin - Madison (Coe, Schneider); Istituto di Psicologia, CNR (Visalverghi); U of Puerto Rico (Phoebus).

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavior Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20895

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.9 | 3.9 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates biobehavioral development through comparative longitudinal study of nonhuman primates, with special emphasis on characterizing individual differences among rhesus monkeys in their behavioral and physiological responses to mild environmental challenges and on determining the long-term developmental consequences for these individuals in different physical and social environments. During FY90 continued longitudinal study of genetic and environmental factors influencing response to challenge yielded findings that both broadened the scope of the phenomena and provided insight as to underlying mechanisms. A new technique for direct intraventricular peptide infusion permitted the assessment of changes in behavior and pituitary-adrenocortical activity in socially living rhesus monkeys following different doses of CRF and CRF anatagonists. Investigations of adolescent rhesus monkeys living in wild settings validated the relationship between individual differences in response to challenge and levels of adrenocortical activity and monoamine turnover previously reported for laboratory-housed adolescent monkeys. A second ongoing field study further related patterns of biobehavioral response to challenge to differential patterns of adolescent male emigration and mortality risk. Completion and staffing of a neonatal nursery permitted the initiation of a new set of studies focusing on rhesus monkey neonatal psychophysiological, neurochemical, attentional, and behavioral predictors of differential response to challenge later in life. Completion of new facilities for housing New World primates made it possible to establish a self-sufficient colony of Cebus apella and to begin parallel studies of factors influencing biobehavioral response to challenge in this primate species.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

Z01 HD 01107-07 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adaptation of Laboratory Reared Monkeys to Field Environments

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I. | S. J. Suomi | Head | LCE, NICHD |
| Other: | S. Boinski | Staff Fellow | LCE, NICHD |
| | R. Bolig | IPA | LCE, NICHD |
| | P. L. O'Neill | Research Psychologist | LCE, NICHD |
| | K. Abbott | Research Psychologist | LCE, NICHD |
| | C. Price | Biologist | LCE, NICHD |

COOPERATING UNITS *(if any)*

OACU, DRS (Bayne); VRB, DRS (Kriete); Dept. of Psychology, U. of Massachusetts (Novak); Dept. of Psychology, Washington State U. (Fragasey).

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Comparative Behavioral Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.9 | 2.6 | 2.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates how rhesus monkeys and other primate species born and raised under different laboratory conditions adapt to placement into naturalistic outdoor environments and compares this adaptation process to that seen in natural settings and in indoor environments that contain specific physical and social features of the monkeys' natural habitat. Adaptation is assessed by examining behavioral repertoires and by monitoring a variety of physiological systems in these subjects, yielding broad-based indices of relative physical and psychological well-being. During FY90 detailed longitudinal comparisons were continued between members of a small multigenerational troop of rhesus monkeys living in a 5-acre outdoor enclosure and members of a second multigenerational group maintained in indoor settings over a comparable period. Detailed analyses of play behavior exhibited by infant and juvenile group members yielded species-normative age and sex differences in the amount and type of play, as well as interactions with the type of housing environment. A new procedure for feeding group-housed rhesus monkeys that resulted in favorable changes in activity cycles and specific behavioral and physiological changes, as well as reducing expense and caretaker staff time, was developed and implemented. Special foraging devices were also utilized to generate new tests of spatial memory and observational learning in both rhesus monkey and Cebus apella groups. A comparative developmental study of vocal behavior of Cebus apella in field and captive environments was initiated, and analysis of vocal behavior and spatial structure in natural populations of two species of squirrel monkeys was continued. Finally, in collaboration with DRS scientists, an inexpensive enrichment device for singly housed primates was developed and tested, resulting in significant, long-term reduction of abnormal behavior patterns.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

Z01 HD 01108-06 LCE

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Comparative Studies of Play Behavior

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | M. Biben | Expert | LCE, NICHD |
| Other: | D. Bernhards | Bio. Lab. Tech. | LCE, NICHD |

COOPERATING UNITS *(if any)*
LCS, NIMH (Winslow)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Brain, Behavior, and Communication

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.2 | 0.3 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The present study is investigating a possible role for morphine and, by extension, endogenous opiods, in the motivation and control of playful behavior in squirrel monkeys. Play is a relatively fragile behavior which is easily disrupted by many stressors and by a variety of drugs, none selectively. Enhancement of play, selective or otherwise, has been a similarly difficult objective to achieve. Morphine is the sole pharmacologic agent with demonstrated ability to increase rates of play, based on work with juvenile rats, where both frequency and content of play are affected.

Squirrel monkeys were chosen for the present studies because our laboratory has established an extensive and exhaustive database on this very playful primate species, giving us a great advantage in detecting deviations from the norm at many levels. Using as subjects pairs of squirrel monkeys who have demonstrated robust play, we found that low doses of peripherally administered morphine (0.5 mg/kg) increased the incidence of play initiation behaviors by the treated animal, indicating increased motivation to play. The duration of play bouts increased by about 30% over baseline levels when one member of the pair received a morphine dose.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01110-03 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Intuitive Parenting of Infants in Comparative Perspectives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | S. J. Suomi | Chief, LCE | LCE, NICHD |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0 | 0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01112-04 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Home and Out-of-Home Care on Child Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M. E. Lamb | Head, SSED | LCE, NICHD |
| Other: | K. J. Sternberg | Research Psychologist | LCE, NICHD |
| | A. Broberg | Visiting Fellow | LCE, NICHD |
| | C. P. Hwang | Visiting Scientist | LCE, NICHD |
| | R. D. Ketterlinus | Staff Fellow | LCE, NICHD |
| | M. Prodromidis | Pre-IRTA Fellow | LCE, NICHD |

COOPERATING UNITS *(if any)*

Center for Human Growth and Development, U of Michigan (Bookstein)

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.2 | 1.0 | 1.2 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A longitudinal study was conducted in Sweden to examine the effects of center day care, family day care, and home care on the development of 145 children recruited at an average of 16 months of age. Multivariate analyses consistently indicated that type of care has no reliable impact on the children one and two years post-enrollment. The quality of home care and the quality of alternative care have equivalent effects on personality maturity, compliance, and emergent social skills with peers and adults. Measures of family social support networks, temperament, and child gender have more modest effects. PLS analyses have also shown that quality of home care was the most important predictor of intellectual competence one and two years after enrollment. Compliance with maternal requests in a task-like situation was also predicted by measures of the mothers' behavior during the observation series.

A second study was focused on the association between infant daycare and security of infant-mother attachment in more than a dozen studies conducted by other investigators. Analyses showed no systematic relationship between enrollment in daycare and security of attachment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01113-04 LCE

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Antecedents, Correlates, and Consequences of Adolescent Pregnancy and Parenthood

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| P.I. | M. E. Lamb | Head, SSED | LCE, NICHD |
| Other: | R. D. Ketterlinus | Staff Fellow | LCE, NICHD |
| | S. H. Henderson | Research Psychologist | LCE, NICHD |
| | K. Nitz | Research Psychologist | LCE, NICHD |
| | G. Virgo | NRSA Fellow | LCE, NICHD |

COOPERATING UNITS (if any)
American Medical Association (Kaplan, Elster); Dept. of Family Studies, Univ. of Tennessee (Barber).

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS:<br>2.4 | PROFESSIONAL:<br>1.3 | OTHER:<br>1.1 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goals of this project are to describe the psychosocial context of adolescent sexual behavior, pregnancy, and parenthood and to explore the longterm effects of early parenthood on mothers, fathers, and children. Our most recent studies have involved data from large nationally representative samples and have addressed two basic sets of questions.

A. Characteristics of adolescent parents. Regardless of race, adolescent parenthood is one symptom of a wide variety of psychosocial problems. Compared with nonfathers and nonmothers of similar ages and backgrounds, adolescent parents are more likely to have a history of involvement with the police, school problems, and substance abuse. A problem behavior syndrome is especially marked among adolescent fathers.

B. Correlates and consequences of adolescent parenthood. Younger adolescent mothers are lighter, gain less weight during pregnancy, seek prenatal care later in their pregnancies, and tend to be from lower socioeconomic classes than are older adolescent and adult mothers. Children of very young adolescent mothers are more likely to be born prematurely and unusually low in birthweight. Among Black and Hispanic mothers, maternal age is not associated with measures of their children's cognitive performance at ages 5-14. For Whites, the children of very young adolescent women have the poorest levels of cognitive performance. Overall, maternal intelligence and socioeconomic status are better predictors of children's performance than is maternal age.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01114-03 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Individual Differences in Physical and Affective Functioning in Infancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M. E. Lamb | Head, SSED | LCE, NICHD |
| Other: | M. P. Fracasso | IRTA Fellow | LCE, NICHD |
| | N. Kimmerly | Pre-IRTA Fellow | LCE, NICHD |
| | A. Scholmerich | Fogarty Fellow | LCE, NICHD |

COOPERATING UNITS *(if any)*

Department of Psychology, U of Maryland (Porges); Department of Psychology, Catholic U (Haynie); Pepin.

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.2 | 1.4 | 0.8 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on individual differences in physical and affective functioning in newborns and the long-term consequences of such differences. One study is concerned with the ways certain psychophysiological and behavioral signs of arousal or irritability (e.g., heart rate, heart rate variability, vagal tone, colic, sleeplessness, crying) in the first months of life are related to measures of the child's temperament, emotional expressiveness, and physiology at later ages.

Preliminary analyses of the data from the initial (5 and 7 month) phases of the study revealed that there was significant temporal stability for the vagal tone, heart rate, and maternal ratings of temperament. Additionally, when compared to infants with moderate vagal tones, 5-month-old infants who had extremely high vagal tone were rated as more fussy and those who had extremely low vagal tone were rated as more unadaptable. At 7 months, the infants with the lowest vagal tone were rated as more unadaptable than the infants with either moderate or with the highest vagal tone scores.

In a second project concerned with interactions between lower-class Hispanic mothers and their infants, SSED staff are studying the effects of maternal behavior, maternal characteristics, family patterns, and infant characteristics upon the subsequent socioemotional development of these Hispanic children. Observing these Hispanic individuals in their homes facilitates the development of culturally-sensitive criteria for evaluating normative social behavior and the development of parent-infant relationships.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 01115-03 LCE

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Effects of Domestic Violence and Evaluation of Children's Testimony

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I. | M. E. Lamb | Head, SSED | LCE, NICHD |
| Other: | K. J. Sternberg | Research Psychologist | LCE, NICHD |
| | S. Horowitz | Research Psychologist | LCE, NICHD |

COOPERATING UNITS *(if any)* Catholic U (Cortes, Krispin, Reiter-Lavery; Hebrew U, Jerusalem (Greebaum, Limor); Jerusalem Minicipality (Garber, Lorey, Saltzman, Zeek); U of MD (Dawud, Sandler); U of N. Dak (Lewensohn); U of Rochester (Cicchetti).

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Social and Emotional Development

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER. |
|---|---|---|
| 1.4 | 0.7 | 0.7 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This program of research comprises two research projects. The goal of the first project is to explore the effects of domestic violence on 8-to 12-year-old children. Four groups of subjects (n=110), each comprising approximately equal numbers of boys and girls, are defined by whether they are: (1) victims of physical abuse by one or both of their parents; (2) witnesses of spousal abuse; (3) both victims and witnesses of domestic violence; and (4) neither victims nor witnesses of domestic violence but have similar backgrounds. Data were obtained from the children, their parents, their homeroom teacher, and their peers. The focus is on the quality of the children's functioning at home, at school, and in the peer group, with attempts made to explore the intrapersonal (temperament; perceptions of responsibility and control) and exogenous (social support) factors that buffer some children while rendering others more vulnerable. Data collection took place in Israel and has been completed; comprehensive data analyses are currently underway. The goal of the second study is to assess the field reliability and validity of a system for eliciting and assessing statements by children who are alleged to have witnessed child sexual abuse. Three sites in Phoenix, Arizona, Marion County, Florida, and Johnson County, Kansas are providing video tapes of child interviews along with case files to establish case facts independent of the statements. Interjudge and test-retest reliability of Statement Validity Analysis (SVA) techniques will be examined. This study will also provide some basic data on children's development of verbal ability and memory for real-life events.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01116-03 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Patterns of Childrearing Across Cultures and Ecologies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | M. E. Lamb | Head, SSED | LCE, NICHD |
| Other: | A. B. Nsamenang | Fogarty Fellow | LCE, NICHD |
| | K. J. Sternberg | Research Psychologist | LCE, NICHD |

COOPERATING UNITS *(if any)* U. Osnabruck, W. Ger. (Keller); U. Goteborg, Sweden (Broberg, Hwang); U. of NC (MacKinnon; U. of MD (Teti, Nakagawa); Inst of Human Sciences, Cameroon (Soh); Hebrew U. (Greenbaum, Rosenthal); Catholic U. (Cortes); U of Leiden, Netherlands (Van Ljzendorn).

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Social and Emotional Development

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.3 | 1.0 | .03 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The work on this project involves a number of studies in a variety of cultures. The overall objective is to explore the ways developmental environments can be described by variations in physical and social ecologies, especially in terms of parental beliefs, values, and practices, and to assess how differences in these domains affect children's development. In the first study, West African parents were interviewed about their perceptions, values, expectations, responsibilities, and practices. These data are being analyzed and will be interpreted in the context of information about variations in developmental niches. Initial results show that urbanicity, generation, and gender independently influence the replacement of traditional endogenous beliefs by those of western origin. In a second study, researchers are attempting to assess specific maternal and child attributions about one another in order to identify the extent to which attributions or expectations shape the way that parents and children interact, with particular focus on the development of aggressive behavior. Third, observational and parent-report measures of infant-mother attachment are being studied in samples of Japanese mothers and infants; assessments of the effects of stress and social support will be used to validate these measures.

| | |
|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 01117-03 LCE |

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Hospitalization Experience: Children's Coping with the Stress of Surgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: M. H. Bornstein Head, CFRS LCE, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Child and Family Research Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

INACTIVE

PHS 6040 (Rev. 1/84) GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01118-03 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Latent Behavioral Effects of Diverse Forms of Caretaking in the First Year of Life

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M. H. Bornstein | Head | LCE, NICHD |
| Other: | Nancy F. Gist | Research Psychologist | LCE, NICHD |
| | Joseph Tal | Statistician | LCE, NICHD |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.1 | 0.9 | 1.2 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This research study is designed to explore the latent effects of different kinds of care in infancy on children's mental and social performance at the preschool level and when the children are finishing their year as first graders. The first-year of school follow-up allows us to compare academic achievement with earlier cognitive status, and to compare children's social behaviors at two points in time straddling entrance to school. This study is the first in a series of investigations preliminary to a large-scale endeavor aimed to document the effects of varied rearing conditions in the first year of life on children's activities and competencies at preschool age and as first graders. Data are being gathered on a homogeneous, low-risk population and include measures of cognitive, social, and behavioral development.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

Z01 HD 01119-03 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Specificity of Mother-Infant Interaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| PI: M. H. Bornstein | Head | LCE, NICHD |
| Other: J. Suwalsky | Research Psychologist | LCE, NICHD |
| P. Ludemann | Research Psychologist | LCE, NICHD |
| C. Rahn | Research Psychologist | LCE, NICHD |
| K. Painter | Research Psychologist | LCE, NICHD |
| M. Fivel | Research Psychologist | LCE, NICHD |
| J. Tal | Statistician | LCE, NICHD |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.5 | 0.9 | 2.6 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates environmental factors that contribute to the development of cognitive and social competencies during the first two years of life. Before children are old enough to enter formal social learning situations, nearly all of their experiences stem directly from interactions they have with their primary caretakers. Four conceptually distinct categories of caretaker-child interactions can be identified: nurturant, material, social, and didactic. These encompass much of the everyday behavior of infants' caretakers. In previous work using samples of convenience, the Principal Investigator linked the latter two types of caretaking to cognitive development in babies. In the present study set, this work is being replicated and extended by focusing on the extent to which three maternal characteristics (age, employment status, and parenthood status) and type of substitute care experienced during mother's employment influence the observed relations between caregiver social and didactic stimulation on the one hand and infant social and cognitive competencies on the other. In addition, in a short-term longitudinal extension of the study, measures of toddler functioning (i.e., play competence, language development, and social adaptation) and maternal behavior (i.e., encouragement of attention to the environment and I.Q.) are being obtained, thereby permitting examination of associations between aspects of mother-infant interaction in early infancy and the development of important aspects of cognitive and social competencies in the second year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01120-03 LCE

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Observations of Parenting and Infant Activity in Different Societies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M. H. Bornstein | Head | LCE, NICHD |
| Other: | S. Toda | Visiting Fellow | LCE, NICHD |
| | P. Ludemann | IRTA Fellow | LCE, NICHD |
| | C. Rahn | Research Psychologist | LCE, NICHD |

COOPERATING UNITS *(if any)* S. Maital, Haifa School Psychological Services, ISRAEL; C. Zingman de Galperin, Universidad de Belgrano, ARGENTINA; H. Azuma, Shirayuri College, JAPAN; M-G. Pêcheux, M. Lamour, Laboratoire de Psychologie du Développement et de l'Éducation de l'Enfant, FRANCE

LAB/BRANCH

Laboratory of Comparative Ethology

SECTION

Section on Child and Family Research

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.8 | 1.7 | 1.1 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experiences in infancy are acknowledged to affect the course and outcome of social and intellectual development in childhood, and they are credited for some of the distinctiveness of culture. More specifically, cross-cultural developmental studies have historically shown that differences in rearing styles typically have implications for children's later cognitive performance and social behavior. Home environments are thought to reflect larger cultural values, beliefs, and customs, and many social theorists have contended that the family generally, and the mother-infant relationship specifically, may be vital to development of the individual and basic to the organization of the culture. As a result, investigators have frequently studied infancy and mother-infant interaction in attempts to address questions about the origins and development of cultural identity. Of course, each society has evolved patterns of child-rearing adjusted to its own special demands.

As one example, it is widely held that Japanese and Americans differ in prominent aspects of their psychological make-ups and that certain social and intellectual distinctions between members of these two cultures arise early in life. Previous study on the nature of infant development in Israeli Kibbutzim determined that many decisive aspects of infant care -- particularly the close ties between infant and mother -- vary markedly from the typical American experience. Contemporary France and America are relatively similar in terms of industrial level, educational attainment, and living standards, yet the two societies differ considerably in terms of history, sociology, and culture. Argentina contrasts middle with extremely poor rearing conditions, all in South American settings virtually unresearched. What differences exist in parenting and in infant activity across these cultures? The purpose of this project is to identify similarities and differences in the childrearing ecologies of Japanese, Israeli, French, Argentine, and American infants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01121-03 LCE

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Parental Activities and Children's Language and Play

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| P.I.: M. H. Bornstein | Head, CFRS | LCE, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Child and Family Research Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

## NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 HD 01122-03 LCE

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Longitudinal Assessment of Children's Mental and Social Abilities

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M. H. Bornstein | Head | LCE, NICHD |

COOPERATING UNITS *(if any)*
Infancy Studies Program, New York University (Tamis-LeMonda)

LAB/BRANCH
Laboratory of Comparative Ethology

SECTION
Section on Child and Family Research

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.8 | 0.6 | 0.2 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The main purpose of this research is to continue an ongoing longitudinal study of mental and social development of a cohort of children observed from 2 months to 4 years. The premise of this research is that learning in childhood occurs primarily within an interpersonal context; thus, a central goal is to describe how diverse infant and maternal behaviors relate to central dimensions of children's social and mental competencies. Children participating in the assessment constitute a longitudinal cohort seen first at 2, 5, 13, and 20 months of age, and finally in a follow-up at 4 years. Major objectives of this longitudinal research are to study stability, continuity, and interaction in mental, social, and emotional development in the first years of life. One notable aspect of the follow-up is a test of materials based on the curriculum-oriented Project Spectrum, organized between the Harvard University School of Education, the Eliot-Pearson Department of Child Study at Tufts University, and the CFRS. Project Spectrum is unique in the United States for its development of a curriculum that goes beyond IQ to assess a wide range of preschool children's interests and capabilities. The 'multiple intelligences' that Project Spectrum's procedures assess include natural science ability, bodily-kinesthetic skills, musical talents, distinctive styles of work, as well as linguistic and logical-mathematical abilities. Because traditional psychometric measures of intelligence at this age typically sample from a narrow range of mental abilities, such measures are limited in terms of the information they provide about the possible relevance of antecedent variables, and they are also restricted in terms of the outcome variables they successfully predict. In contrast, the Project Spectrum Field Assessment Battery samples from a wide range of preschoolers' capabilities and interests and thus affords richer opportunities to respond to many theoretical and pragmatic questions that surround the central issue of stability in mental development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00040-15 LTPB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Statistical and Mathematical Studies of Molecular Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | P. J. Munson | Head | UBM, LTPB, NICHD |
| Others: | D. Rodbard | Head | LTPB, NICHD |
| | K. Oerter | Medical Staff Fellow | LTPB, DEB |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Unit on Biostatistical Methodology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 10892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | 1 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigations into analysis of hormone time series led to the development of a new technique and computer program named PULSEFIT. This model-based approach to characterizing circulating hormone measurements has been applied to measurement of LH in normal and orchiectomized males and in pre- and peripubertal males and females. We found that the most commonly used measure of pulsatility, namely number of pulses in the series, has a large statistical variability (50 to 100%). We defined a new pulsatility index which has considerably lower statistical variability and displays greater statistical power for discerning differences between groups within each of these studies. This new method provides better discrimination between the patterns of gonadotropin secretion during various stage of puberty than was previously appreciated. This method should be useful in the analysis and reanalysis of studies of pulsatile hormone secretion.

We had previously developed a semiparametric approach to the analysis of families of curves, which combines the advantages of a parametric method (e.g., relative potency estimates) with the flexibility and versatility of nonparametric methods, such as smoothing cubic splines. However, the method was often slow to converge and sensitive to choice of initial estimates. Continuing theoretical studies of this method (FLEXIFIT) has led to a dramatic reduction in the size and complexity of the algorithm, accompanied by substantial increases in speed. The FLEXIFIT minimization algorithm is now comparable in speed to more routine, four-parameter logistic modeling. This enhancement comes at the cost of a negligible loss of optimality of the goodness of fit. However, the reduction of time and complexity promise to have a considerable impact on the practicality of the method.

The FLEXIFIT method has been applied successfully to data from radioimmunoassays (RIA), bioassays, enzyme-linked immunosorbent assays (ELISA), and to the calibration curves which arise in polyacrylamide and agarose gel electrophoresis of proteins and nucleic acids.

| | |
|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00165-15 LTPB |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation and Characterization of Macromolecular and Cellular Particles

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. Chrambach | Head | LTPB, NICHD |
| Others: | N. Rampino | Biotechnology Fellow | LTPB, NICHD |
| | D. Tietz | Visiting Scientist | LTPB, NICHD |

COOPERATING UNITS *(if any)*
Laboratory of Developmental Immunology (R. Schneerson, T. A. Cramton)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Macromolecular Analysis

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.7 | 0.7 | 00 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) DNA of several hundred kb in size, labelled by a fluorescent intercalating dye, was shown to exhibit a) a distribution of condensed or linear conformations depending on buffer concentration during electrophoresis (1 V/cm, 25°C); b) a variable conformation for a single DNA molecule during migration, changing from a roughly spherical to a V-shaped to an extended and back to a roughly spherical conformation; c) a migration rate varying from a maximal value for the spherical molecule down to zero for the symmetric V-shaped species; d) stretching of the V-shaped species while its migration rate decreases or increases. This migration behavior is interpreted as entanglement of an elastic molecule on the gel fiber, elastic stretching during entanglement and elastic disentanglement and recoil concomitant with free migration until the cycle of entanglement and migration repeats itself. The entanglement can be at single or multiple locations on the DNA molecule. Elastic stretching at excessive field strength leads to breaking of the molecule.

2) Size, surface net charge and relative abundance of components (of a given size and charge) were determined for 21 polydisperse particle populations of protein-bacterial coat carbohydrate conjugates. Aim of the characterization was to correlate relative immunogenicity of vaccine preparations directed against meningitis with these physical properties.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00171-14 LTPB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electrophoretic Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. Chrambach | Head | LTPB, NICHD |
| Others: | D. Tietz | Visiting Scientist | LTPB, NICHD |
| | J. Pospichal | Visiting Fellow | LTPB, NICHD |
| | N. Rampino | Biotechnology Fellow | LTPB, NICHD |
| | M. Deml | Courtesy Associate | LTPB, NICHD |
| | L. Orban | Guest Researcher | LTPB, NICHD |

COOPERATING UNITS *(if any)*

Biomedical Engineering and Instrumentation Program, NCRR, NIH (A. Aldroubi, M. Unser); Polysciences Inc. (D. Halpern and T. R. Ittyerah).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Macromolecular Analysis

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.4 | 3.4 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1) The information regarding DNA size and conformation inherent in the rate of change of electrophoretic mobility with gel concentration (Ferguson plot) was derived from pore gradient gels oriented at a right angle to the direction of polymerization (transverse pore gradient gels). This approach avoids the previous assumption of the proportionality between size and migration distance, overcomes limitations in the number of bands and is non-laborious. 2) Agarose gel electrophoresis in discontinuous buffer systems is operative at low DNA load (1 microgram/squ.cm) and at 0.04 M ionic strength. These conditions avoid previous perturbed gel patterns. 3) Free mobility of proteins was measured by polyacrylamide gel electrophoresis in the presence of 0.4% agarose, exploiting the absence of significant sieving effects at this concentration. This allows one to identify a molecule by size and charge, to pinpoint the optimal way of separating it from the species migrating as neighboring bands and to computer simulate migration. 4) Apparatus for electrophoresis with continuous monitoring through epifluorescence microscopy was constructed. It allows one to visualize, and take measurements on, single DNA molecules up to several hundred kb in size during electrophoresis. 5) Apparatus for electrophoresis in sieving media consisting of liquid polymer solutions was constructed. Size separation of polystyrene particles in the range of 125 to 1,000 nm radius was achieved. Mobilities in that size range were found to be proportional to particle size. 6) Continuous optical scanning of gels during electrophoresis was compared with conventional detection of gel patterns at a single point of observation. This comparison aims at finding the minimal throughput time and the maximal number of components separable per unit length of gel, and at validating, or invalidating, the assumed advantages of continuous pattern observation. 7) Glutaraldehyde crosslinked polyvinylalcohol with molecular weights of 25,000 and 650,000 was evaluated as a gel electrophoretic medium. 8) Exportable programs for analysis of gels on the basis of the extended Ogston model at single concentration, 2-D agarose gels (ELPHOFIT) and linear pore gradients (GRADFIT) were written.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00189-09 LTPB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Computer Programs for Analysis of Laboratory and Clinical Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. Rodbard | Head | LTPB, NICHD |
| Others: | P. Munson | Head | UBM, LTPB, NICHD |
| | K. Oerter | Medical Staff Fellow | LTPB, NICHD |

COOPERATING UNITS *(if any)*

National Cancer Institute (O. W. McBride); Consorcio Negri Sud, Chieti, Italy (V. Guardabasso); University of Modena, Modena, Italy (A. Genazzani)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.25 | 0.25 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed an improved method for analysis of the calibration curves which arise in agarose and polyacrylamide gel electrophoresis of linear duplex DNA fragments using continuous or pulsed-field conditions. We employ a "four parameter logistic" model, which introduces one more parameter than was used previously in a rectangular hyperbola model. This method provides better goodness of fit, better precision, and reduced bias. The method is widely applicable, especially in studies of physical mapping of the human genome and genetic linkage analysis. A new program, DNAFIT, has been developed to implement this method for routine analysis with semi-automated methods involving digitizing tablets. The method provides improved quality control for routine laboratory analyses and also facilitate choice of optimal gel concentration for a desired molecular weight range.

We have developed improved methods for detection and characterization of episodic hormone secretion, and to estimate the instantaneous rate of hormone secretion using deconvolution analysis. These methods have been applied to several clinical investigations of the dynamics of LH, FSH, prolactin, ACTH, cortisol, and beta-endorphin.

New methods have been developed to evaluate coincidence of pulses in two hormonal time series. These include the concept of "specific concordance" as a function of threshold levels and adjustable lag times, and also uses the Kappa statistic.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01400-08 LTPB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Applications of Stable Isotopes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. L. Yergey | Head | LTPB, NICHD |
| Others: | N. Vieira | Biologist (Tech.) | LTPB, NICHD |
| | S. Abrams | NRSA | LTPB, NICHD |

COOPERATING UNITS *(if any)* Lab. Math. Biol., NCI (D. Covell); U. Tenn., (R. Goans); Dept. Ped. U. MO Med. Sch., Columbia, MO (L. Hillman); Dept. Endoc., Mayo Clinic (R. Eastell); Cin. Child Hosp. (B. Specker); USDA, Beltsville, MD (C. Veillon, P Moser); Dept. of Nutr., U.Conn. Storrs (L. Allen); Children's Hosp. NMC (A. Fletcher); U. TX. Med. Cntr., Galveston (D. McCormick).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION

NICH, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.5 | 1.5 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☑ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continuing work using calcium stable isotopic tracers for the measurement of absorption, distribution and excretion of calcium have led to a number of clinically significant findings. 1) Calcium metabolism in 5 adolescents with anorexia nervosa (age 17.0±2.2 yrs, wt 36.1±6.7 kg) showed reduced absorption, increased urinary calcium excretion and negative calcium retention compared to healthy adolescent females (age 14.0±1.7 yrs., wt 57.1±11.5 kg - mean ± SD). Patients with anorexia nervosa consumed 1650 mg/day calcium and absorbed 215±55 mg/day, excreted 252±73 mg/day in urine leading to a net retention of -36±74 mg/day. The healthy girls consumed 1000 mg/day Ca and absorbed 427±53 mg/day, excreted 143±41 mg/day in urine and had a net retention of 284±94 mg/day. The differences between the patients with anorexia nervosa and healthy subjects may be a consequence of the elevated cortisol levels in the former [See project Z01 HD 01400-07 LTPB]. 2) Calcium metabolism studies in 6 premature infants (1.4±0.3 kg BW, 19±12 days age at study) fed premature infant formula and consuming 214±15.1 mg/kg/day Ca had absorbed 138.1±33.7 mg/kg/day, excreted 2.8 mg/kg/day in urine and endogenous fecal excretion of 17.1 mg/kg/day and had net retention of 118.3±38.2 mg/kg/day. These values demonstrate calcium retention comparable to in utero levels. 3) Studies of calcium distribution in Cynomolgus monkeys (ages 35-38 mos) before and after 6 wks of testosterone administration showed significant increases in growth velocity (0.107±0.003 to 0.353±0.20 mm/wk, p=0.001), mass of calcium in the rapidly turning over pool (22.3±10.3 to 63.7±24.3 mg. p<0.01) after administration of testosterone. This hormone may increase the availability of calcium in the rapidly turning over pool and thereby alter growth rate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01401-08 LTPB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biological Applications of Thermospray Chromatography/Mass Spectrometry

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. Yergey | Head | LTPB, NICHD |
| Others: | N. Esteban | Visiting Associate | LTPB, NICHD |
| | D. Vicchio | Staff Fellow | LTPB, NICHD |
| | M. Sakairi | Adjunct Scientist (CRADA) | LTPB, NICHD |

COOPERATING UNITS *(if any)*

Children's Hospital Nat'l. Med. Center (T. Silber); U. Conn. (L. Allen); DEB, NICHD (T. Loughlin, F. Casorla, K. Karalis); NCI P-Navy MOB (J. Mulshine, T. Treston).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Metabolic Analysis and Mass Spectrometry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.25 | 3.25 | 0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☑ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Continuing use of stable isotope dilution liquid chromatography/mass spectrometry (LC/MS) have produced a number of significant findings:

1) Measurement of cortisol levels and cortisol production rates a) the value of urinary free cortisol in anorexia nervosa (n=5) averages 97±46 μg/day vs normal values of 42±17 μg/day. Morning serum cortisol levels in the anorexia nervosa patients averaged 32±12 μg/dl (normal 10-20μg/dl). These data suggest that, despite high calcium intakes, significant abnormalities in calcium metabolism exist in adolescents with this eating disorder [See project Z01 HD 01400-08 LTPB]. 2) The earlier findings of daily FPR being significantly lower than accepted values has raised issues of appropriate therapies. Bioavailability studies using oral hydrocortisone and a tri-dutero tracer to determine the appearance of cortisol in plasma indicate absorption of 56±10% in 4 patients with adrenal insufficiency. These data may be used to determine appropriate oral replacement levels of cortisol. 3) Measurement of the endogenous production rate of 25-OH vitamin $D_3$ (25OH-D) has been accomplished using 6, 19, 19-$^2H_3$-25-OH-vitamin $D_3$ as a tracer. Using a single pool model and bolus infusion of tracer at ~5% of the plasma pool level of preliminary results yield a value for the half life of 25D of 11±1 days (n=5). Earlier studies using radio-labelled tracers have yielded values of the half life of 25OH-D of about 20 days in humans and have suggested that, in rats, the value varies with dietary fiber. We undertook the measurement of the half life of 25OH-D in humans along with the investigation of the effects of dietary fiber on calcium absorption and 25OH-D metabolism. Preliminary results show a) half life that is reduced by about 50% from earlier results, and b) no perturbation of the 25OH-D metabolism by high levels of dietary fiber.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01404-07 LTPB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Characterization of Opioid and Peptide Receptors in Brain and Peripheral Tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. Rodbard | Head | LTPB, NICHD |
| Others: | G. Z. Zhou | Visiting Associate | LTPB, NICHD |
| | P. J. Munson | Mathematical Statistician | LTPB, NICHD |

COOPERATING UNITS *(if any)*

Institute for General & Experimental Pathology, University of Innsbruck, Innsbruck, Austria (S. Schwarz)

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.20 | 0.20 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have used quantitative ligand binding studies to characterize complex, heterogenous receptors for hormones and neurotransmitters in brain and other tissues.

We have evaluated the hypothesis, that homocysteic acid (HCA) is a naturally occurring neurotransmitter for the N-methyl-D-Aspartate (NMDA) type of the glutamate receptor. The NMDA receptor is allosterically coupled to an ion channel, to which the drug MK-801 binds with high affinity and specificity. We examined the binding of [3H]-MK-801 to brain membranes which were thoroughly washed to remove endogenous amino acids and other small ligands. Repletion of L-glutamate, or L-homocysteic acid, resulted in parallel dose response curves for [3H]-MK-801 binding. This effect was potentiated by low concentrations of glycine, a known allosteric modulator of this system. More than 20 other amino acids were ineffective, demonstrating the specificity of L-HCA. A moderate degree of stereoselectivity was demonstrated. HCA appears to be selective for the NMDA receptor, and not effective at the kainic acid or quisqualate receptors. Together with data (e.g., electrophysiological) from other laboratories, these studies support the hypothesis that L-HCA may be an endogenous neurotransmitter at excitatory amino acid receptors.

The effects of several monovalent and divalent cations on the binding of ligands to the "sigma" and phencyclidine (PCP) receptors have been examined in detail. These two types of receptors show differential sensitivity to ions.

The sigma and PCP receptors of membranes from rat and guinea pig brain have been characterized using quantitative ligand binding studies with self-and cross displacement studies, dose response surfaces and extensive computer modelling. We demonstrate that sigma and PCP sites are distinct, and that the PCP sites can be resolved into high and low affinity forms for both rat and guinea pig, while the sigma receptor can be resolved into two forms in the guinea pig.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 01406-02 LTPB |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Interactions between mammary epithelium, adipocytes, and extracellular matrix

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

|  |  |  |  |
|---|---|---|---|
| PI: | J. C. Calvo | Visiting Scientist | LTPB, NICHD |
| Others: | D. Rodbard | Head | LTPB, NICHD |
|  | C. Rondinone | Visiting Fellow | LTPB, NICHD |
|  | S. Chernick | Consultant | LTPB, NICHD |

COOPERATING UNITS *(if any)*
NIDR, BRB (M. Yanagishita), NIDDKD, LCDB (C. Londos, J. Blanchette-Mackie); and NHLBI, CM (V. Manganiello).

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | 1 |  |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Development of the breast at the time of thelarche and during pregnancy and lactation is under endocrine, paracrine and autocrine control. Differentiation of the lobuloalveolar structure requires interaction between epithelium and connective tissue stroma. To study the complex interactions between these cell types, we sought to develop a simplified model system consisting of cultures of normal mouse mammary gland epithelium and 3T3 and 3T3-L1 preadipocytes. We observed: 1) exposure to conditioned media from normal mouse mammary gland inhibits proliferation of the same kind of cells, suggestive of the presence of a "mammastatin" as recently reported in the human; 2) conditioned media from 3T3 or 3T3-L1 cells stimulate proliferation and triglyceride accumulation by 3T3-L1 cells, a positive autocrine effect; 3) culture of normal mouse mammary gland in an insert separated by a membrane from an underlying layer of 3T3-L1 cells stimulates growth of mammary epithelium; 4) a factor secreted by normal mouse mammary gland strongly inhibits differentiation of preadipocytes into adipocytes. This is shown by inhibition of triglyceride accumulation when 3T3-L1 preadipocytes were co-cultured with mammary epithelium, when they are exposed to the media from normal mouse mammary gland through inserts in the tissue culture dishes, or by exposure to conditioned media derived from the mammary epithelial cells. A dose response curve is obtained. Activity is retained by passage through a filter with 30 kDa cutoff, and is lost with heating, consistent with a protein factor. This activity may be attributable to one or more known growth factors or it may represent a new protein factor. We are also examining the effects of sex steroids on the proliferation and differentiation of preadipocytes. Preliminary results indicate that progesterone stimulates differentiation and triglyceride accumulation. Studies are underway to evaluate whether this effect is mediated by the progesterone or by the glucocorticoid receptor; 5) differentiation of the preadipocytes is accompanied by marked stimulation of the rate of synthesis and release of a large chondroitin sulfate proteoglycan with approximate molecular weight of 970,000 with a core protein of 370,000 molecular weight with 12 carbohydrate side chains of approximately 48,000 molecular weight. This protein appears to be closely related to versican. This system for study of proteoglycan synthesis and secretion is unusually sensitive to hormonal stimulation with glucocorticoids and inhibitors of cAMP phosphodiesterase.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01405-06 LTPB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Computer Programs to Aid Intensive Insulin Therapy for Type-I Diabetes Mellitus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. Rodbard | Head | LTPB, NICHD |
| Others: | M. Berger | Volunteer Researcher | LTPB, NICHD |

COOPERATING UNITS *(if any)*
Yale University School of Medicine (P. Miller, M. Berger); Kantonsspital Basel, Basel Switzerland (M. Berger)

LAB/BRANCH
Laboratory of Theoretical and Physical Biology

SECTION
Section on Theoretical Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: .20 | PROFESSIONAL: .20 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed computer programs for the simulation of plasma insulin and glucose dynamics after subcutaneous injection of insulin. The program incorporates a pharmacokinetic model to calculate the time courses of plasma insulin for various combinations of popular preparations (Regular, NPH, Lente, Ultralente). Utilizing a pharmacodynamic model describing the dependence of glucose dynamics on plasma insulin and glucose levels, the program can predict the expected time course of plasma glucose in response to a change in carbohydrate intake, insulin dosage, timing or regimen. A set of typical parameters has been obtained by analysis of data from the literature. Several computer simulations have been generated to evaluate the effects on insulin and glucose profiles of systematically changing insulin regimen, dose, timing of meals or timing of preprandial insulin administration. The program can be used to explore on a theoretical basis the impact of various factors associated with glycemic control. As an educational tool the program provides a suitable environment for demonstration of the combined or isolated effects of insulin and diet on glycemia. Further, we have developed programs for analysis of blood glucose values obtained by persons performing self-monitoring of blood glucose. These programs provide several novel features, including an animated computer "movie" showing how the 24 hour glucose profile changes longitudinally with time. For a patient with a stable pattern of intake, exercise and endogenous (hepatic) glucose production, one can predict the "ideal insulin profile." We use a simplified, linearized model, to permit one to optimize the doses of insulin for any one of eight insulin regimens (involving up to four injections per day), to most closely approximate this ideal insulin profile, and to select the best regimen. This program (Glucoject, Version 2) should be useful for teaching purposes and for decision support. Another version, CADMO, provides an English language interpretation of the GLUCOJECT output, to facilitate use by paramedical personnel and physicians. Preliminary results also indicate the potential utility of electronic exercise monitors to automatically capture data regarding constancy of patient lifestyle, to help in the interpretation of data from the glucose memory meters.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01407-01 LTPB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

Mechanisms of Receptor Mediated Signal Transduction

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | T. Costa | Visiting Scientist | LTPB, NICHD |
| Others: | Y. Ogino | Visiting Fellow | LTPB, NICHD |
| | P. Munson | Head | LTPB, NICHD |
| | D. Rodbard | Head | LTPB, NICHD |

COOPERATING UNITS (*if any*)

NINDS, Lab. of Mol. & Cell. Neurobiol. (C. Fraser); NIDDK, MPB (A. Spiegel); Univ. of Aquila Med. Sch., L'Aquila, Italy (S. Adamo).

LAB/BRANCH

Laboratory of Theoretical and Physical Biology

SECTION

Section on Theoretical Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | 1 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

We have analysed the mechanism of coupling between receptors and their transduction proteins (guanine nucleotide binding protein, or G proteins) in intact membranes. Classical receptor theory predicts that antagonists passively occupy the binding site of the receptor without altering its conformation, and that receptors are not active in the absence of ligand.

We have demonstrated that some opioid and b-adrenergic antagonists act by inducing, upon binding, a state of the receptor which has lesser affinity for the G protein than the unliganded receptor. We have called these ligands "antagonists with negative efficacy", because they disrupt the spontaneous coupling between receptor and G protein in the membrane, and In vivo, may produce effects that are opposite to those of the agonist. We found that receptors can activate G proteins in membranes even in the absence of ligands; this spontaneous interaction is selectively inhibited by sodium ions with a range of concentrations very close to that of the intracellular environment, suggesting that sodium may be a physiological modulator of signals transmitted by G protein-coupled receptors.

The spontaneous receptor activity, the effect of sodium, and negative antagonism can all be explained by a ternary-complex model of receptor-G protein interaction, according to which, the association and dissociation of these two molecules in the membrane is governed by a stability constant that is altered upon binding of the ligand. The ability of a ligand to either enhance or diminish this stability constant determines whether such ligand has positive or negative efficacy. Sodium reduces the affinity between receptor and G protein but not the ability of the ligand to perturb their equilibrium. Thus, sodium is an allosteric regulator of signal transduction, which selectively filters hormone-independent "noise" without suppressing hormone-dependent stimuli.

We also studied the interaction between multiple receptors stimulating phosphoinositide turnover and activation of protein kinase C in human neuroblastoma cells that are capable of spontaneous interconversion between a neuroblast and glial phenotype. We found that phorbol ester-induced desensitization of receptor-mediated responses exhibits different patterns in the two cell phenotypes, suggesting that protein kinase C regulates molecules involved in signal transduction by distinct mechanisms in the two phenotypes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00047-21 LDN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Studies of Neurons and Other Cell Types

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. Brenneman | Pharmacologist | LDN,NICHD |
| Others: | R. Avidor | Visiting Fellow | LDN,NICHD |
| | I. Gozes | Visiting Scientist | LDN,NICHD |
| | S. McCune | NRSA Fellow | LDN,NICHD |
| | D. Warren | Bio. Lab. Tech. | LDN,NICHD |
| | M. Voigt | Guest Researcher | LDN,NICHD |
| | A. Fatatis | Guest Researcher | LDN,NICHD |

COOPERATING UNITS *(if any)*

Lab. Cell Biol., NIMH (L.Eiden, D.Agoston); Biol Psych Br, NIMH (J. Glowa); Lab. Mol. Biol. (M. Voigt); Dept. Physiol/Univ. College, Cardiff (G. Foster); Dept.Pathol/Ohio State Univ. (R. Mervis); Peptide Design (J. Hill); Dept. Organic Chem/Weizmann Inst.(M. Fridkin); Dept. Neurol/Univ. of Kansas (B. Festoff); Div. Cell. & Neuropathol/Karolinska Inst. (M. Schultzberg). Dept. Chem. Pathol/Tel Aviv Univ. (I. Gozes).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Neurochemistry

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 2.6 | PROFESSIONAL: 2.0 | OTHER: 0.6 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Neuropeptides were studied as both regulators of activity-related neurodevelopment and as substances that were themselves regulated by electrical activity. Primary cell culture preparations from the mammalian central nervous system were employed. Vasoactive intestinal peptide was shown to act as a secretagogue for the following from rat cortical astrocytes: an interleukin-1-like substance and protease nexin I. A reversible down regulation of mRNA for VIP was observed in spinal cord cultures that were electrically blocked with tetrodotoxin. This activity-related gene regulation of VIP was attenuated with active phorbol ester or exogenous VIP.

A novel antagonist to VIP, when administered to developing rats, was found to produce deficits in complex motor behaviors without delays in the occurrence of developmental milestones. Dystrophic neurons were observed within deep layers of cerebral cortex of developing rats which had been treated with the VIP antagonist. In mature rats, the VIP antagonist was shown to produce cognitive impairment as assessed by performance in the Morris water maze. The regional specificity for the antagonist was demonstrated in that VIP receptors located in lymph nodes, superior cervical ganglion, gut, lung and pancreas were not responsive, whereas those in spinal cord, hippocampus and cerebral cortex were potently inhibited.

Northern blots of mRNA were probed with random-primed DNA from the alpha-2-D, alpha-2-A and alpha-1-B adrenergic receptor cDNAs. A unique regional and ontogenic expression was observed for these three subtypes of alpha receptors present in rat brain and other tissues.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00056-15 LDN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biosynthesis, Processing & Secretion of Neuropeptides & Pituitary Peptide Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Y. Peng Loh | Head | LDN, NICHD |
| Others: | Nigel Birch | Visiting Assoc | LDN, NICHD |
| | Mogens Fenger | Visiting Fellow | LDN, NICHD |
| | Theodore Friedman | Medical Staff Fellow | LDN, NICHD |
| | Oi-Lian Kon | Guest Researcher | LDN, NICHD |
| | Howard Tracer | NRC-Biotech Assoc | LDN, NICHD |
| | Winnie Tam | Microbiologist | LDN, NICHD |
| | Joon Kim | Stay-in-School | LDN, NICHD |

COOPERATING UNITS *(if any)*

Laboratory of Cell Biology, NIMH (M. Brownstein); IMBICE, La Plata, Argentin (F. Estivariz)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Cellular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.75 | 4.5 | 1.25 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pituitary secretory vesicle enzymes involved in the processing of pro-opiomelanocortin (POMC, pro-ACTH/endorphin) and pro-vasopressin were studied. A 70,000 mol. wt. paired basic residue specific prohormone converting enzyme (PCE), has recently been characterized as a unique calcium activated aspartic protease. Cloning of this enzyme is in progress. Studies were conducted to uncover mechanisms underlying the differential processing of POMC in the anterior and intermediate pituitary and brain. In vitro experiments show that calcium can regulate the extent of cleavage of POMC to smaller products. Thus, intravesicular calcium concentrations may regulate the quantitative differences in processing of β-LPH to β-endorphin in the anterior versus the intermediate lobe. Another finding was that 'O'glycosylation at Thr45 of N-POMC inhibited PCE from cleaving the Arg-Lys50 bond to yield N-POMC1-49 and Lys50γ3MSH. Since the non-'O'glycosylated form of N-POMC is found in intermediate but not anterior lobe, this post-translational modification of POMC may account for the presence of N-POMC1-49 and Lys50γ3MSH only in intermediate lobe. The signal involved in the targeting of POMC into the regulated secretory pathway was also investigated. Truncated POMC/CAT constructs were transfected into the endocrine AtT-20 cells. Analysis of the intracellular localization of the expressed POMC-CAT fusion protein analyzed by immunofluorescence histochemistry using anti-CAT, and secretion studies with forskolin stimulation indicate that the N-terminal 25 amino acids of the prohormone is sufficient to target POMC into the regulated pathway. Binding studies suggest the presence of a receptor in secretory vesicle membranes which preferentially binds N-POMC1-77 at an acidic pH and may be involved in the sorting process. Our previous work showed that during salt-loading, anterior pituitary POMC mRNA and hypothalamic AVP mRNA levels were increased. Recently, a dramatic increase during salt-loading, of a polyA truncated form of AVP mRNA in the neural pituitary was discovered. This increase persisted with hypothalamic stalk transection suggesting that the AVP mRNA is locally synthesized, most likely in the pituicytes.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00064-14 LDN |
|---|---|

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurobiologic Studies of Neurons and Glia in Cell Culture

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | P.G. Nelson | Chief | LDN, NICHD |
| Others: | E.A. Neale | Head, Unit on Cell Biology | LDN, NICHD |
| | C. Yu | Visiting Associate | LDN, NICHD |
| | R.D. Fields | Senior Staff Fellow | LDN, NICHD |
| | P. Lin | Visiting Fellow | LDN, NICHD |
| | S. Fitzgerald | Biologist | LDN, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 7 | 4 | 3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A compartmental tissue culture system has been used to control neuronal activity, to measure the strength of synaptic connections between defined neuronal populations and test hypotheses as to the mechanisms involved in activity-dependent synaptic plasticity. Postsynaptic electrical activity, NMDA receptor activation and intracellular calcium levels interact in determining the long term changes in synaptic efficacy produced by chronic stimulation of synaptic inputs.

Axonal outgrowth (growth cone activity) from DRG neurons is quickly blocked by electrical activation (phasic stimulation is more effective than tonic). With prolonged stimulation (hours), the growth cone can become accommodated and resume outgrowth. Electrical stimulation increases intracellular calcium in growing axons and their growth cones. The increase is transient and the calcium buffering capacity of axons appears to be regulated, in part, by electrical activity.

BayK 8644 reliably augments high-voltage activated (HVA) calcium currents while ω-conotoxin produces a moderate decrease in these currents in cultured mouse spinal cord (SC) and DRG neurons. BayK 8644 augments synaptic interactions between these cell types in a minority of cases (no change in most), while ω-conotoxin is without detectable effect (ω-conotoxin eliminates synaptic transmission at the frog neuromuscular junction). Our results suggest a high degree of heterogeneity with regard to the calcium channels responsible for neurotransmitter release.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00094-20 LDN

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pineal Regulation: Environmental and Physiological Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D.C. Klein | Head | LDN, IRP, NICHD |
| Other: | Tamar Babila | Visiting Fellow | LDN, IRP, NICHD |
| | Nicolas Schaad | Visiting Fellow | LDN, IRP, NICHD |

COOPERATING UNITS *(if any)*

Georgetown U. (M.A.A. Namboodiri); NEI (T. Shinohara); NIDDK (A. Speigel); U. of Giessen (H. Korf)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.4 | 1.0 | 0.4 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the environmental and physiological regulation of the pineal gland, exclusive of transmembrane and intracellular regulatory mechanisms (see Z01 HD 00095-20 LDN). The pineal gland is part of the melatonin rhythm generating system, a neural circuit which includes a circadian clock in the suprachiasmatic nucleus (SCN); the SCN is reset and entrained by light acting through the eye. Several new areas of research have been initiated in this program within the past year. One involves $Na^+,K^+$-ATPase. It has been discovered that the pineal contains three subtypes of this enzyme. Analysis of neural and photic regulation of the levels of these enzymes has revealed that one of the high affinity forms of the enzyme is selectively depressed following surgical denervation of the gland. This may reflect either a neuronal localization of this form of the enzyme, or the existance of a neural mechanism which regulates levels of this enzyme. Another area of interest is proteins involved in transmembrane signal processing. These include G proteins, MEKA and the S-antigen. These studies indicate that MEKA and the S-antigen are absent before birth and appear rapidly during the first postnatal week in the rat. In contrast, $G\beta$ is present at adult levels prior to birth. In the adult, it has been found that the amount of MEKA and the phosphorylation state of this enzyme are regulated by environmental lighting. Total MEKA increases at night; phosphorylation also increases at night in the dark and the protein is rapidly dephosphorylated by exposure to light.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00095-20 LDN

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pineal Regulation: Transsynaptic and Intracellular Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D.C. Klein | Head | LDN, IRP, NICHD |
| Other: | J.L. Weller | Chemist | LDN, IRP, NICHD |
| | B. White | Senior Staff Fellow | LDN, IPR, NICHD |
| | S. Donohue | Biotechnology Fellow | LDN, IPR, NICHD |
| | P. Roseboom | Biotechnology Fellow | LDN, IRP, NICHD |
| | Y. Li | Visiting Fellow | LDN, IRP, NICHD |
| | T. Babila | Visiting Fellow | LDN, IRP, NICHD |
| | N. Schaad | Visiting Fellow | LDN, IRP, NICHD |

COOPERATING UNITS *(if any)*

Georgetown U. (M.A.A. Namboodiri); NEI (T. Shinohara); AFRRI (J. Halperin); Yale U (R. Levenson); U. of Giessen (H. Korf); NIDDK (A. Spiegel); NCI (W. McBride); NIDR (M. Iadarola); U. of Oregon (J. Adelman); NINCDS (P. Rowe); NIMH (E. Ginns)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.8 | 5.7 | 1.1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies neurochemical signal transduction and the control of tissue specific gene expression, using the pineal gland as a model. Efforts are directed at determining the details of the chemical and ionic components of transmembrane signalling processing and in the neural and tissue specific regulation of gene expression. Signal transduction in this system is of special interest because cAMP and cGMP are regulated by dual receptor mechanisms which appear to interact at the level of regulation of adenylyl and guanylyl cyclases. One leg of these pathways activates these enzymes via GTP binding regulatory proteins, similar to GSα. This leg is controlled by β-adrenergic or VIP receptors; activation of this leg produces only partial stimulation of cAMP and cGMP accumulation. Activation of the other leg is via $\alpha_1$-adrenergic receptors. This activates protein kinase C which acts, perhaps on the regulatory or catalytic proteins, to increase the activation of adenylyl and guanylyl cyclase. Activation of protein kinase C occurs as a result of an increase in $[Ca_2^+]$i and in diacyglycerol production by phospholipase C. In addition, in the regulation cGMP, there appears to be a strong requirement for activation of phospholipase A and for an increase in $[Ca_2^+]$i. Current activities are focused on the role of MEKA in signal transduction. MEKA is a 33 kDa substrate of protein kinase A and exists as a cytoplasmic complex with GSβγ. Immunoprecipitation is being used to isolate and study the phosphorylation states of GSα, adenylyl cyclase and guanylyl cyclase complexes. In the area of the neural and developmental control of gene expression, advances have been made in purifying N-acetyltransferase and hydroxyindole-O-methyltransferase, and in isolating cDNA clones coding for these enzymes. A human hydroxyindole-O-methyltransferase cDNA clone has been sequenced and the gene has been located on the pseuodoautosomal region of the Y chromosome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00704-06 LDN

PERIOD COVERED

October 1, 1989 - September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Tetanus Toxin Effects and Localization in Neurons

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | E.A. Neale | Head | LDN, NICHD |
| Others: | H.I. Trenchard | IRTA Fellow | LDN, NICHD |
| | L.C. Williamson | IRTA Fellow | LDN, NICHD |
| | L.M. Bowers | Biologist | LDN, NICHD |
| | S.C. Fitzgerald | Biologist | LDN, NICHD |

COOPERATING UNITS *(if any)*

Division of Bacterial Products, Center for Biologics Evaluation and Research, Food and Drug Administration (W.H. Habig)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Cell Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.6 | 1.5 | 0.1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Tetanus toxin elicits an electrophysiologic response in primary neuronal cultures similar to that observed in the nervous system in situ. A biochemical assay for neurotransmitter release was developed in order to define the phases of toxin action corresponding to the physiologic response. Excitatory and inhibitory neurotransmitter pools were radiolabeled, and transmitter release was assayed at various times after toxin exposure. Relative to control cultures, release of the inhibitory neurotransmitter, glycine, was unaffected during the latent period, and was inhibited during the phases of convulsant activity and electrical quiescence. Release of the excitatory neurotransmitter, glutamate, was unaffected during the latent period, slightly increased during convulsant activity, and inhibited during quiescence. These patterns of release parallel increases and decreases in the frequency of recorded excitatory and inhibitory postsynaptic potentials, and indicate that the phase of convulsant activity can be defined by differential transmitter release and electrical quiescence, by inhibition of transmitter release.

Electron microscopy of synaptic terminals showed progressively fewer structures associated with synaptic vesicle release and recycling, and increased numbers of synaptic vesicles adjacent to release sites, with time of toxin exposure. These results indicate that the final toxin-induced blockade of synaptic transmission appears related to an inability to synaptic vesicles to fuse with the presynaptic release site.

At the time when synaptic transmission between mature (i.e., more than four weeks in culture) spinal cord neurons is blocked by tetanus toxin, the synaptic connection between sensory and spinal cord neurons is not affected. Whereas toxin bound to the recorded spinal cord neurons can be visualized immunohistochemically, the sensory neurons appear unstained. Thus, the failure to block the sensory-spinal cord neuron connection may be related to the inability of toxin to bind to mature sensory neurons.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00705-09 LDN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Functional organization of the nerve terminal

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Dr. James T. Russell | Head | LDN, NICHD |
| Others: | Dr. Gilbert Chin | Senior Staff Fellow | LDN, NICHD |
| | Dr. Kemal Payza | IRTA Fellow | LDN, NICHD |

COOPERATING UNITS *(if any)*

LB, NINCDS (E. Stanley); LMB, NIMH (D.M. Neville); LB, NIDDK (D. Sevrese); Inst. of Med. Physiology-C, University of Copenhagen, Denmark (N.A. Thorn & E. Engberg); University of Houston (D.H. Malin); University of Southern California (G. Augustine).

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Neuronal Secretory Systems

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.0 | 3.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The research program is directed towards studying the biochemistry and physiology of the nerve terminal with particular emphasis on intracellular ion transients, modulation of secretion and mechanism of exocytotic secretion. The neurohypophysial nerve terminals are used as the model to study the importance of ion channels and receptors on initiation and modulation of secretion. These include studies on secretion of vasopressin and oxytocin from isolated neurosecretosomes, and calcium ion transients in the nerve terminals. Neurosecretosomes are maintained under tissue culture conditions and this preparation is suitable for studies of the mechanism of secretion, and high resolution microscopy. Neurosecretosomes respond to depolarizing stimuli with markedly elevated secretion. Calcium ionophores also cause secretion in the absence of applied depolarization. The inactivation of hormone release from neurosecretosomes was found to be independent of membrane depolarization. Tetanus toxin in nM concentrations blocks secretion of both hormones induced by depolarizing stimuli, as well as by calcium ionophores. Secretion of both hormones was inhibited by the opiate k-receptor agonists and somatostatin. This receptor-mediated modulation of secretion at the nerve terminal is under investigation. Analogues of FMRFamide related peptides have been developed to study their receptors in mammalian and squid CNS. A study of synaptic vesicle associated proteins in the squid resulted in the characterization of low molecular weight GTP-binding proteins and synaptobrevin. A cell-free system and a permeabilized cell model are being developed to study the exocytotic process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

ZO1 HD 00707-06 LDN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacological Studies of Synaptic Transmission In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.L. Mayer | Visiting Scientist | LDN, NICHD |
| Others: | M. Benveniste | NRC fellow | LDN, NICHD |
| | D. Patneau | IRTA Fellow | LDN, NICHD |
| | C. Winters | Chemist | LDN,NICHD |

COOPERATING UNITS *(if any)*

Section on Instrumentation, Research Services Branch, NIMH (B.M. Smith and S. S. Hsiao)

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Section on Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.0 | 3.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Unit investigates the mechanism of action of excitatory amino acids in the vertebrate CNS, utilizing cell culture and electrophysiological techniques. A fast perfusion system is used for concentration jump application of agonists and antagonists to nerve cells under voltage clamp. A series of 15 amino acids, including the transmitter candidates L-glutamate, L-aspartate and L-homocysteate, as well as L-sulfocysteine and quinolinic acid, agonists of neuropathological interest, were examined for agonist activity at AMPA and NMDA receptors. L-glutamate and its analogues were mixed agonists with activity at both receptors, while L-aspartic acid was a selective NMDA receptor agonist. The potency range for NMDA receptor activation was 1000-fold; $EC_{50}$ values were from 2.3 $\mu$M (L-glutamate) to 2.3 mM (quinolinate). Activation of rapidly desensitizing responses at AMPA receptors required approximately 100-fold higher concentrations of agonists. At NMDA receptors equilibrium dose response curve analysis suggests that the binding of two molecules of agonist is required to activate ion channel gating. Kinetic analysis of the action of competitive NMDA receptor antagonists reveals sigmoidal kinetics, well fit by a two binding site model. In contrast to results from previous analysis using less complex kinetic models, two site microscopic association and dissociation rate constants for antagonists are good predictors of equilibrium potency. Experiments in progress use this analysis to study structure-activity relationships for novel NMDA receptor antagonists. The block of responses to kainate by the AMPA receptor agonist quisqualate shows a competitive interaction at equilibrium, but with slow recovery kinetics. The IC50 for quisqualate block of kainate responses is similar to the concentration of quisqualate required for half block of rapidly desensitizing responses at AMPA receptors. This could occur is AMPA receptor agonists were partial agonists at the same receptor at which kainate produced non desensitizing responses.

| | |
|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>ZO1 HD 00708-06 LDN |

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morphologic Studies of Neuronal and Non-neuronal cells in CNS Cell Cultures

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Elaine A. Neale | Head | LDN, NICHD |
| Others: | Linda M. Bowers | Biologist | LDN, NICHD |

COOPERATING UNITS *(if any)*

Laboratory of Neurophysiology, NINDS, NIH (T.J. Smith, Jr.); Division of Bacterial Products, Center for Biologics Evaluation and Research, Food and Drug Administration (W.H. Habig and J.L. Halpern).

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Cell Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.4 | 0.6 | 0.8 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of morphologic complexity has been measured using tetanus toxin immunohistochemistry and analysis of fractal dimension in dissociated cultures of mouse spinal cord. Increases in complexity with time are non-linear, the most rapid development occurring over the initial 24 hr after plating.

Functional rFragment C of tetanus toxin, cloned and expressed after using the polymerase chain reaction, appears to be a suitable alternative to tetanus toxin or toxin-derived fragment C for studies of its role in toxin binding and internalization, and for use in immunohistochemistry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00709-04 LDN

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Prevention of Neuronal Deficits Associated with AIDS.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D. Brenneman

| | | | |
|---|---|---|---|
| Others: | S. Fitzgerald | Biologist | LDN, NICHD |
| | I. Gozes | Visiting Scientist | LDN, NICHD |
| | S. McCune | NRSA fellow | LDN, NICHD |
| | J. Buzy | Guest Worker | LDN, NICHD |

COOPERATING UNITS *(if any)*
Div. of Neuropath., Ohio State Univ. (R. Mervis); Peptide Design, L.P. (J. Hill, C. Pert and M. Ruff); Psychol. Dept., American Univ. (L. Panlilio); Clin. Neuroendocrinol. Branch (J. Glowa); Dept. Oncology, Long Island Jewish Hosp. (F. Siegal); Head Trauma Unit, Walter Reed Army Hosp. (A. Martin, A Salazar); U.S.-Japan Biomed. Research Lab., Tulane Univ. (A. Arimura).

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Unit on Neurochemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.2 | 1.8 | 0.4 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rat neonates treated systemically with purified gp120, the external envelope protein of the human immunodeficiency virus, exhibited retarded development of complex motor behaviors and widespread dystrophic changes in neuronal morphology. The dystrophic neurons of the cerebral cortex were characterized by reduced dendritic arbor and coarse, thickened dendrites with abnormal spines.

Co-administration of peptide T with gp120 prevented or attenuated the retardation in behavioral development. A Sholl analysis of dendritic spread indicated that peptide T prevented the 25% loss observed in cortical neurons from animals treated with gp120 alone.

Adult rats given gp120 daily by intracerebroventricular cannula showed impaired acquisition of learning in the Morris water maze test. In saline-injected rats, performance progressively improved over a 15 days of training while animals given gp120 did not improve.

PACAP38, a VIP-like neuropeptide isolated from ovine hypothalami, was found to prevent gp120-induced death in murine hippocampal cultures. The potency of PACAP38 was 100-fold greater than VIP in preventing neuronal death associated with gp120.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00710-02 LDN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Characterization of Glutamate Receptor Expression in Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. Buonanno | Head | LDN, NICHD |
| Others: | V. Gallo | NATO Fellowship | LDN, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Molecular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Excitatory amino acid receptors (EAARs) are the site of action of different psychoactive drugs, and have been implicated in simple forms of learning and memory. A natural ligand of EAARs is glutamate, which activates three pharmacologically distinct sites known as the kainate, AMPA, and NMDA receptors. Kainate activates the opening of a channel selective for sodium and calcium. In collaboration with Dr. R. Wenthold's group, the properties of a cDNA coding for a frog kainic acid binding protein (KABP) were studied. Results obtained using a *Xenopus* oocyte expression system, in conjunction with two-electrode voltage clamp, showed that the transcripts coded by the frog KABP cDNA can not form functional channels in injected oocytes. The conclusion from these results are that the cDNA codes for a putative subunit of the kainate receptor that cannot form functional channels in absence of other structural components, or that it codes for a KABP with a function not presently understood. In mammals kainic acid receptors (KARs) are particularly abundant in the cerebellum; present at higher densities in the molecular and granular cell layers. We have chosen to study KAR expression in the cerebellum because the cell types, organization, and development of this area have been extensively studied. Oligonucleotides, corresponding to the sequence of a recently cloned rat kainate receptor subunit (KAR1), were used to screen a rat cerebellum cDNA library at low stringency. A novel 3.9 kb cDNA clone was obtained that spans the entire coding region; this clone was called KAR2. The deduced amino acid sequence of the KAR2 cDNA codes for a protein that is 60% homologous with KAR1. Most of the homology resides in the portion of the protein predicted to code for membrane spanning regions. Northern blot analysis was used to determine the regional distribution of KAR2 mRNAs. The KAR2 transcripts are more abundant in cerebellum than in hippocampus, cortex, thalamus, and spinal cord. Preliminary studies using cerebellar tissue cultures suggest that KAR2 transcripts are present in granule cells and absent in cerebellar astrocytes; cerebellar astrocytes have been shown to respond to kainate application. Experiments are in progress to study the expression of KAR2 during development. In the future we plan to study the molecular mechanisms underlying the cell-specific and developmental expression of the KAR2 gene, as well as, other genes coding for EAARs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 HD 00711-01 LDN

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Transcriptional Regulation of Synapse-Specific Genes in Skeletal Muscle

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. Buonanno | Head | LDN, NICHD |
| Others: | R. Eftemie | IRTA | LDN, NICHD |
| | L. Lautens | Visiting Fellow | LDN, NICHD |
| | R. Beers | Biologist | LDN, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Developmental Neurobiology

SECTION

Unit on Molecular Neurobiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20982

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Myogenin and MyoD are proteins that bind to the regulatory regions of a battery of skeletal muscle genes and activate their transcription during muscle differentiation. We found that both proteins interact with the enhancer of the nicotinic acetylcholine receptor (nAChR) α subunit, a gene that is regulated by innervation. Gel retardation and DNA footprinting were used to identify the regions in the enhancer bound myogenin and MyoD. The regions contained a consensus sequence for the MyoD binding site. The functional role of the binding sites was assessed by transfecting myotubes with a construct containing the α subunit enhancer linked to the gene chloramphenicol acetyl transferase (CAT). Point mutations in the binding sites specifically obliterated expression of CAT in the transfected muscle cells. Since the nAChR gene is regulated by myogenin and MyoD, we decided to study how the expression of these factors is regulated during myogenic differentiation, and during innervation. The levels of myogenin and MyoD transcripts were found to decrease in mouse hind muscle during the perinatal period, a time when innervation is taking place. This decrease is due to innervation, since denervation of adult skeletal muscle lead to a dramatic increase in the levels of both transcripts. Interestingly, the responses of myogenin and MyoD to innervation and denervation, precede the changes in nAChR mRNA levels. Muscle "electrical activity", in absence of nerve, was found to down-regulate the levels of myogenin and MyoD transcripts. Since myogenin and MyoD are *trans*-regulatory factors that activate the expression of a large battery of skeletal muscle genes during differentiation, it is interesting to speculate that their repression during innervation could lead to the selective down-regulation of synaptic genes. We have also focused in identifying the *cis*-elements that regulate transcription of the nAChR γ subunit gene. Two regions that regulate the transcription of the γ subunit gene during myoblast differentiation were identified. One region enhances the expression of the CAT reporter gene driven by the γ subunit upstream regulatory sequence, and another site represses its expression. Experiments are in progress to determine if these regulatory sequences function as classical enhancers and silencers, and if they are regulated by myogenic factors related to myogenin or MyoD.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 01202-3 LDN |
|---|---|

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Expression and Function of Neuropeptides During Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Y. Peng Loh | Head | LDN, NICHD |
| Others: | May Wong | Senior Staff Fellow | LDN, NICHD |
| | Adrian Rius | Visiting Fellow | LDN, NICHD |
| | William Hayes | NRC/Biotech Assoc. | LDN, NICHD |
| | Judith Hewitt | NRC/Biotech Assoc. | LDN, NICHD |

COOPERATING UNITS *(if any)*
Department of Biochemistry, St. Louis, Univ (C. Coscia)

LAB/BRANCH
Laboratory of Developmental Neurobiology

SECTION
Section on Cellular Neurobiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.5 | 4.5 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to study the molecular and cellular mechanism regulating the developmental expression of three neuropeptide genes coding for the pro-opiomelanocortin (POMC), thyrotropin releasing hormone TRH and the pro-[met]enkephalin family of peptides, and to investigate the role of these peptides in development, particularly in the central nervous system (CNS). Two model vertebrate systems: the frog (Xenopus laevis) and mouse were used. The temporal and spatial expression of POMC, TRH and [met]enkephalin mRNA during development have been mapped by in situ hybridization histochemistry. The first appearance of POMC mRNA in the mouse CNS was in the region of the presumptive arcuate nucleus, at embryonic day 10-1/2 (E10-1/2), and in the anterior lobe and intermediate lobe of the pituitary at E12-1/2 and E14-1/2 respectively. Immunocytochemical and biochemical analyses showed that the POMC was expressed at E10-1/2 but processing of the prohormone began only a day later, at E11-1/2. The major embryonic POMC-derived products were ACTH$_{1-17}$, des acetyl $\alpha$-MSH, and $\beta$-endorphin$_{1-31}$ and $\beta$-endorphin$_{1-27}$ in brain and ACTH$_{1-39}$ and $\beta$-endorphin$_{1-31}$, indicating some differences in processing between embryos and adult. Characterization of opiate receptors in embryonic mouse brain revealed the first expression of the mu receptors which bind specifically to $\beta$-endorphin$_{1-31}$, but not the truncated or acetylated forms of the peptide, at E12.5. These studies indicate that the POMC system is functional as early as E12.5 in the mouse CNS. In the frog, migrating pituitary precursor cells were found to express mature POMC mRNA at the same time that they reach the infundibular wall at stage 28. The first POMC expressing cells in the CNS was found in embryonic forebrain (the posterior diencephalon) at stage 31 and they were juxtaposed to the first POMC cells found in the pituitary primordium. The finding of the first brain and pituitary POMC cells juxtaposed at the juncture of the diencephalic and infundibulum walls suggest brain-pituitary interactions in the setting up of this system. The frog POMC and pro-enkephalin genes including the 5' upstream regulatory region have been cloned and sequenced. Analysis of the 5' upstream region of the POMC gene have identified the promoter sequence and a cyclic AMP regulatory element. Work is now in progress to identify the elements responsible for tissue-specificity expression and the trans acting factors involved in activating these genes during development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00066-20 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Control Mechanisms in Temperate Bacteriophage Lambda

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I. | R. Weisberg, Head | | LMG/NICHD |
| Others: | K. Cam | Visiting Fellow | LMG/NICHD |
| | E. Gersnon | Summer Student, 1990 | LMG/NICHD |
| | L. Dorgai | Visiting Associate | LMG/NICHD |
| | J. Oberto | Visiting Associate | LMG NICHD |
| | N. Ramaiah | IRTA | LMG/NICHD |
| | S. Sloan | Microbiologist | LMG/NICHD |
| | M. Ditto | IRTA | LMG/NICHD |

COOPERATING UNITS *(if any)* Institute of Cancer Research; Columbia University, NY (Max Gottesman); Department of Biochemistry, Tel Aviv University, Israel (Ezra Yagil); Laboratory of Molecular Biology, NIMH (Howard Nash).

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Microbial Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.7 | 3.7 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A. The integrase proteins of phages λ and HK022 are site specific recombinases that are closely related in structure and function, but recognize different nucleotide sequences in the core regions of their substrates, the attachment sites of the two phages. In order to locate the parts of the proteins that discriminate between the substrates, we constructed and analyzed the substrate specificity of a large set of λ/HK022 hybrid proteins. These proteins were produced by selecting crossovers between short regions of identical sequence shared by the λ and HK022 *int* genes. We found that exchange of segments upstream of amino acid residue 202 or downstream of residue 283 in λ integrase had little or no effect on substrate specificity, and we conclude that the "discrimination domain" lies between these residues.

B. We have begun a mutational analysis of *E. coli* Integration Host Factor (IHF) with the goal of determining which regions of this small DNA binding and bending protein are directly involved in specific sequence recognition and DNA bending. We have so far isolated and characterized a small set of mutations that interfere with the function of the wild type protein ("dominant negative" mutations). We have also found, contrary to previous indications, that IHF is abundant in exponentially growing cells: the concentration is comparable to that of ribosomal proteins.

C. We have continued our studies of the action of the HK022 Nun protein, a sequence specific transcription termination factor. We have shown that partially purified Nun protein specifically strongly and specifically depresses the synthesis of β-galactosidase in a coupled transcription-translation system, and that Nun action requires the presence of a functional *nut* site upstream of the *lac*Z gene. The requirement for a *nut* site agrees with previous *in vivo* studies. Surprisingly, the effect of Nun in this system appears to be mainly post-transcriptional: Nun depresses *lac*Z message much less strongly than β-galactosidase. We are pursuing this suggestion that Nun may act on more than one level.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00067-22 LMG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Integration of Macromolecular Synthesis in Escherichia coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M. Cashel | Section head | LMG:NICHD |
| Others: | M. Kalman | Visiting Scientist | LMG:NICHD |
| | H. Xiao | Visiting Fellow | LMG:NICHD |
| | P. Jones | IRTA Fellow | LMG:NICHD |
| | D. Gentry | Guest Worker (NRC Fellow) | LMG:NICHD |
| | H. Murphy | Chemist | LMG:NICHD |

COOPERATING UNITS *(if any)*
Gad Glaser, Dept. Cellular Biochemistry, Hadassah Medical School, Jerusalem, Israel

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Molecular Regulation

INSTITUTE AND LOCATION
NICHD/NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.6 | 2.6 | 1.00 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall purpose of this project is to understand cellular mechanisms which coordinate gene expression during transitions between balanced growth and nutritional impoverishment. We remain particularly interested in the regulatory roles played by the structurally and metabolically related guanine nucleotides pppGpp and ppGpp that are analogs of GTP and GDP, respectively, but bear a 3'-pyrophosphate. These unusual nucleotides have been implicated as a regulatory signal in these adjustments.

Past annual reports have described the relA and spoT genes involvement in the synthesis and degradation of (p)ppGpp. We also described manipulating (p)ppGpp levels at will. Last year's annual report described a first glimpse of the properties of cells lacking detectable ppGpp. This year, we have probed further into RNA polymerase rpoBC suppressors of one component of the pleiotropic phenotype associated with ppGpp-dependent functions. We have sequenced and devised a way to exploit the remaining gene (gpp) in the (p)ppGpp metabolic pathway so as to manipulate the relative abundance of pppGpp and ppGpp. This should allow us to dissect nucleotide-specific effects in the future. Prokaryotic genes sharing transcriptional regulation in an operon often show related functions. Accordingly, we have explored the downstream region of the spo operon at 83 min and sequenced, localized, and partially characterized recG, gltS, and probably gltC genes. We have also discovered that there are two genes involved in gpp function rather than one; gppA and gppB. The gppA gene was found to share an operon with a new gene, we call rhlB which shares sequence homology with the so-called DEAD Box family of RNA helicase genes. We have also isolated three more putative rhl genes with PCR techniques, sequenced portions of each, and mapped all five.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00068-19 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Factors Influencing Genetics Transcription-Initiation and Termination

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

| | | | |
|---|---|---|---|
| PI: | R.J. Crouch | Research Chemist | LMG:NICHD |
| Others: | D.-Y. Shin | Visiting Fellow | LMG:NICHD |
| | D. Ennis | Senior Staff Fellow | LMG:NICHD |
| | E. Kalman | Visiting Associate | LMG:NICHD |
| | M. Kavuru | Pre-IRTA | LMG:NICHD |
| | L. Bundara | Microbiologist | LMG:NICHD |
| | D. Tang | Biological Aid (SIS) | LMG:NICHD |
| | P. Barr | Summer IRTA | LMG:NICHD |

COOPERATING UNITS (if any)

LMG, NICHD (J. Levin); Columbia University, New York (W. Hendrickson).

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Formation of RNA

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.04 | 3.07 | 1.97 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Analysis of the crystal structure of E. coli RNase H indicates a protein of a five-stranded $\beta$-sheet surrounded by five $\alpha$-helices. Conserved, essential carboxylic acid amino acids probably bind $Mg^{++}$ and thereby initiate an interaction between the enzyme and the substrate. These negatively charged, conserved amino acids converge with other conserved residues on one face of the molecule. Retroviral and yeast RNase HI have additional domains which may affect the location and/or function of their RNases H. The RNase H domain of yeast functions without the remainder of the protein whereas the retroviral RNase H exhibits a requirement for most of the polymerase domain for maximal activity.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00069-18 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Genetics of Mammalian Retrovirus Replication

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J.G. Levin | Research Biochemist | LMG, NICHD |
| Others: | Y.-X. Feng | Visiting Scientist | LMG, NICHD |
| | J. Guo | Visiting Fellow | LMG, NICHD |
| | K. Post | Biologist | LMG, NICHD |
| | T.K. Ng | SIS | LMG, NICHD |

COOPERATING UNITS *(if any)* NICHD-LMG (R. Crouch); ABL Basic Research Program, NCI-FCRDC (A. Rein); NCI-LEC (D. Hatfield); PRI-FCRDC (M. Zweig); CBER-DV (C. Marcus-Sekura)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Viral Gene Regulation (Developmental Biology Section)

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.0 | 2.7 | 1.3 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects
  ☐ (a1) Minors
  ☐ (a2) Interviews

☐ (b) Human tissues

☒ (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of this project is to define the molecular mechanisms involved in the replication of mammalian retroviruses and in particular, to understand the factors which influence the regulated expression of viral genetic information. Studies are being carried out with the murine leukemia virus system. Current interest is focused on the functional relationship between the polymerase and RNase H domains of reverse transcriptase (RT). Mutant and wild-type RT proteins were expressed in *E. coli* and then purified. The expressed proteins include (i) full-length RT; (ii) an RT (ΔSX) with a deletion of 127 residues immediately upstream of the RNase H domain; (iii) an RT (ΔH) missing the entire RNase H domain; and (iv) a chimeric protein having the viral polymerase domain fused to *E. coli* RNase H. All of the proteins, with the exception of ΔH, have RNase H activity. The full-length, chimeric, and ΔH proteins have high levels of polymerase activity, whereas the polymerase activity of the ΔSX protein is reduced 200-fold. Interestingly, the ΔSX RNase H activity is also reduced and the characteristic preference for Mn$^{2+}$ over Mg$^{2+}$ is lost. This result shows that despite the existence of two separate domains in RT, a mutation in one domain can influence the other. Efforts are now underway to further characterize the RNase H activity of these proteins using substrates which resemble viral intermediates formed during reverse transcription. In other work, translational control of viral gene expression is being investigated by studying readthrough suppression of the UAG codon at the MuLV *gag-pol* junction. When this UAG is mutated to UAA or UGA, both of these termination codons are suppressed in vitro and in vivo with the same efficiency as UAG. The amino acids inserted in vitro in response to UAA and UGA have been identified: UAA, like UAG, directs the sole incorporation of glutamine,whereas UGA directs the incorporation of arginine, cysteine, and tryptophan. The MuLV suppression system is highly specific and other known UAG and UGA suppressor tRNAs (leucine and serine, respectively) although present in our system, are not functional. This suggests that the "codon context" at the *gag-pol* junction not only affects the efficiency of suppression, but also determines which tRNAs will be used for suppression. Experiments to elucidate the identity and role of context are in progress.

| | |
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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 01001-08 LMG |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Organization and Expression in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | I. Dawid | Head | LMG:NICHD |
| Others | N. Bhatia-Dey | Visiting Fellow | LMG:NICHD |
| | D.-H. Huang | Visiting Associate | LMG:NICHD |
| | A. Mazo | Visiting Scientist | LMG:NICHD |

COOPERATING UNITS *(if any)*
Jim Kennison, LMG:NICHD

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 2.0 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Two genes involved in the determination of segment identity in Drosophila were studied: female (1) sterile homeotic (fsh), and trithorax (trx). The fsh gene is required maternally for proper differentiation of body pattern, whereas trx acts predominantly in the zygote. Mutations in fsh and trx interact strongly, leading to high levels of homeotic transformations of a bithorax type. To ask whether this interaction is specific, a large fraction (about 20%) of the entire genome was tested for dosage-dependent maternal interactions with trx or zygotic interactions with fsh. In addition to several regions that gave effects of low penetrance, one chromosomal region was found that showed strong interactions. This region, defined by a deletion, contains the ash-1 gene that is known from previous work to interact with fsh and trx, and the kohtalo gene, suspected of such interactions. The paucity of regions in the genome affecting the expression of fsh and trx suggests that the synergism between mutations in these two genes is based on highly specific interactions.

A molecular analysis of the trx gene has been continued with the expression of segments of the very large open reading frame (3759 amino acids) as fusion proteins in bacteria and yeast. In this way the entire trx protein has been expressed. As predicted from the sequence it was shown that certain regions of the protein are capable of binding zinc in vitro. This finding is compatible with but does not establish the hypothesis that the trx protein is a member of the zinc finger family of transcription factors. Several trx fusion proteins have been used for the production of antisera that will be employed in the characterization of the trx protein.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 01002-08 LMG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Expression During Embryonic Development of Xenopus Laevis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| PI: | I.B. Dawid, Head | LMG:NICHD |
| Others: | J. Greene, NRC Fellow | LMG:NICHD |
| | N. Bhatia-Dey, Visiting Fellow | LMG:NICHD |
| | P. Good, IRTA Fellow | LMG:NICHD |
| | M. Taira, Visiting Scientist | LMG:NICHD |
| | M. Rebbert, Chemist | LMG:NICHD |
| | H. Mahy, Student Volunteer | LMG:NICHD |

COOPERATING UNITS *(if any)* LC, DCE, NCI (A. Roberts and M. Sporn)

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.0 | 4.8 | 1.2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mesoderm induction is the first cell interaction in the amphibian embryo, and likely in other vertebrate embryos as well. Therefore the mechanism of this induction is of considerable interest. Members of two classes of growth factors are known to be effective in this event: fibroblast growth factor (FGF) and transforming growth factor beta (TGF-$\beta$). We have shown that TGF-$\beta$ isoforms differ in their inducing capacity; earlier work identified TGF-$\beta$2 as a mesoderm inducing factor while TGF-$\beta$1 and $\beta$5 are inactive. Recently we demonstrated that TGF-$\beta$3 is a more potent inducer than other known TGF-$\beta$ isoforms. In addition we have confirmed the reports of others that activin A, a distant homolog of TGF-$\beta$, is the most potent inducer identified so far. To study the mechanism of induction we isolated cDNA clones encoding the FGF receptor from Xenopus. FGF-R mRNA is present in early embryos when induction takes place, and disappears from uninduced ectoderm as it differentiates into epidermis.

A nervous system-specific gene encoding a putative protein component of nuclear ribonucleoprotein complexes has been isolated and characterized. This gene, named nrp-1, is expressed in the developing brain of Xenopus tadpoles in the ventricular zone where cell proliferation takes place. We suggest that nrp-1 expression is required for the proliferation of cells in the nervous system. In a search for additional RNP protein genes in Xenopus we isolated a close relative of the nrp-1 gene, a relative of the ubiquitously expressed A1 hnRNP protein gene, and the frog homolog of the A2/B1 hnRNP protein gene. Tissue-specific RNP proteins may be involved in the differential control of RNA metabolism in development, and may thus be components of a system of gene regulatory mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 01004-07 LMG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Amino Acid Biosynthetic Genes in Saccharomyces cerevisiae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: A.G. Hinnebusch, Research Microbiologist, (All listed personnel LMG/NICHD)

| | | | | |
|---|---|---|---|---|
| Others: | P. Miller | NRC Fellow/Sen Staff Fel. | C. Moehle | NRC Fellow |
| | N. Williams | Guest Researcher | M. Cigan | NRC Fellow |
| | E. Hannig | IRTA Fellow/Sen Staff Fel. | M. Ramirez | Visiting Fellow |
| | M. Foiani | Visiting Fellow | T. Dever | NRC Fellow |
| | J. Bushman | Pre-IRTA Fellow | B. Freeman | Biologist |
| | R. Wek | IRTA Fellow | | |
| | J.P. Abastado | Visiting Fellow | | |

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Laboratory of Molecular Genetics

SECTION
Unit on Molecular Genetics of Lower Eukaryotes (Section on Developmental Biology)

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 13.0 | 11.33 | 1.67 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

GCN4 is a transcriptional activator of amino acid biosynthetic genes in S. cerevisiae that is regulated at the translational level by upstream open reading frames (uORFs). uORFs 3 and 4 repress GCN4 translation under nonstarvation conditions; uORF1 allows ribosomes to traverse uORFs 3 and 4 and translate GCN4 in amino acid-starved cells. We have strong evidence that ribosomes which translate GCN4 have previously translated uORF1, resumed scanning and bypassed uORFs 2-4. This occurs under starvation conditions because reinitiation of translation is inefficient in these circumstances. GCD factors are required for translational repression of GCN4. GCD1 and GCD2 appear to be involved in general translation and are physically associated with initiation factor -2 (eIF-2). In addition, mutations affecting eIF-2 subunits have a Gcd$^-$ phenotype. These findings suggest that antagonism of general initiation factors under starvation conditions leads to reduced reinitiation at uORFs 2-4 and consequent increased translation of GCN4. Antagonism of GCD factors requires the GCN1, GCN2 and GCN3 proteins. GCN3 is present in the GCD1-GCD2-eIF-2 complex and thus appears to act directly in modulating its function. This is consistent with the isolation of gcn3 mutations that derepress GCN4 expression in the absence of GCN1 and GCN2. GCN2 is a protein kinase that contains a domain homologous to histidyl-tRNA synthetases, which prompted the idea that its kinase function is activated by uncharged tRNA in starved cells. This suggestion is supported by the isolation of high copy-number plasmids containing tRNA genes that suppress a leaky gcn2 mutation. Increased GCN2 kinase function is thought to antagonize the GCD1-GCD2-GCN3-eIF-2 complex and thereby stimulate translation of GCN4. A defective protein phosphatase gene (GLC7) was also isolated as a dominant suppressor of the leaky gcn2 mutation, suggesting that a balance between phosphorylation (by GCN2) and dephosphorylation (by GLC7) of initiation factors determines the efficiency of translational reinitiation. Ribosomal protein (rp) genes are repressed under amino acid starvation conditions. This repression is mediated by the binding site for the regulatory protein RAP1. RAP1 activation of rp genes is modulated by amino acid levels at a post-translational level, by a mechanism independent of GCN1-GCN4. Thus, separate pathways exist for regulating amino acid biosynthesis and ribosome biogenesis in response to amino acid availability.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01005-03 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Cellular Proliferation and Diversity in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J. Kennison | Senior Staff Fellow | LMG, NICHD |
| Others: | A. Felsenfeld | NRC Biotech Fellow | LMG, NICHD |
| | B. Judge/Brizuela | Biologist (Tech) | LMG, NICHD |
| | J. Ballard | SIS | LMG, NICHD |

COOPERATING UNITS *(if any)*

Department of Biology, University of California, Santa Cruz, California (John Tamkun); LMC, NCI, NIH (Richard Kahn); Department of Biology, Indiana University, Bloomington, Indiana (Thomas Kaufman); LMG/NICHD/NIH (Igor Dawid)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Section on Developmental Biology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.5 | 2.0 | 1.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Genes involved in the determination of segmental identity in Drosophila melanogaster have been identified on the basis of genetic interactions with homeotic mutations, mutations already known to affect the process. Mutations in previously-identified homeotic genes have been recovered, as well as mutations in new homeotic genes. One of the known homeotic genes, the trithorax locus, and four of the new homeotic genes (the genes kismet, brahma, moira, and osa) have been characterized in more detail. The kismet gene has been shown to encode a post-transcriptional regulator, coactivator, or function as a downstream target for the homeotic gene Antennapedia. The remaining four genes are transcriptional regulators of Antennapedia. The kismet gene has been cloned and spans >60 kb of genomic DNA. Two partially-overlapping transcripts (8 and 17 kb in length) appear to be derived from alternative promoters. The brahma gene has also been cloned and encodes a single 5.5 kb transcript present at all developmental stages. DNA sequence analysis of cDNA clones indicates that the brahma protein may include a conserved region found in three other proteins, the Drosophila fsh protein, the yeast spt7 protein, and the human CCG1 protein. The Drosophila brahma and fsh genes both exhibit similar genetic interactions with two other homeotic genes, suggesting a functional role for the conserved protein domain.

The transcription unit adjacent to the brahma gene has been shown to encode a member of the superfamily of small GTP-binding proteins. As the predicted protein sequence is most similar to the yeast and mammalian ADP-ribosylation factor proteins (ARFs), the gene has been named arf-related (arl). The purified Drosophila protein binds guanine nucleotides and hydrolyzes GTP, but is unable to substitute for ARFs in an in vitro ARF functional assay. An embryonic-lethal arl mutation was isolated. arl is the first member of the superfamily of small GTP-binding proteins demonstrated to be essential in a multicellular eukaryote.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01006-02 LMG

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*

Protein-Nucleic Acid Interactions in Vertebrate Embryogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | |
|---|---|
| PI: | T. Sargent, Research Microbiologist (All listed personnel LMG/NICHD) |
| Others: | E. Jonas, Visiting Associate |
| | A. Snape, Visiting Associate |
| | P. Mathers, NRC Biotech. Assoc. |
| | R. Winning, Guest Researcher |
| | L. Shea, Biologist |
| | S. Marcus, HHMI/MCPS Guest Researcher |

COOPERATING UNITS *(if any)*

Clinical Endocrinology Branch, NIDDK (Sheryl Sato)

LAB/BRANCH

Laboratory of Molecular Genetics

SECTION

Unit on Vertebrate Development (Section on Developmental Biology)

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER. |
|---|---|---|
| 4.86 | 3.75 | 1.11 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The aim of this project is to identify and characterize factors that regulate gene expression in vertebrate embryos as an approach to understanding the mechanisms that control early development. The primary model system is the transcriptional regulation of an epidermal keratin gene, XK81A1, in the amphibian (Xenopus laevis) embryo. The cis-regulatory elements of this gene have been mapped by introducing mutated DNA constructs into frog embryos. A positive transcription factor, KTF-1, has been identified which binds to a specific regulatory site and stimulates the activity of the keratin promoter. KTF-1 has been partially purified by affinity chromatography, and its characterization is being continued.

Other potential regulatory elements in the keratin promoter are being mapped by various approaches, including PCR-amplified in vivo footprinting. By this latter method several potentially interesting sites of DNA-protein interaction have been identified, including sequences resembling the binding sites for the mammalian transcription factors AP-1 and AP-2. Molecular clones for these factors are available and can be used to clone Xenopus homologs.

In collaboration with Dr. Sheryl Sato (Clinical Endocrinology Branch, NIDDK) the investigation of the posterior-specific putative zinc finger protein called Xpo (Xenopus posterior) is being continued. Xpo can be induced in ectoderm by a combination of mesoderm inducer (such as Activin A or Fibroblast Growth Factor) and retinoic acid. The possible role of Xpo in controlling axis formation is being investigated by injection of synthetic mRNA into fertilized eggs.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 01007-01 LMG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structure, Function and Genetics of Nucleic Acid-Binding Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| PI: | I.B. Dawid, Head | LMG:NICHD |
| Others: | G. Michaels, Adjunct Scientist | LMG:NICHD |
| | P. Bray, IRTA Fellow | LMG:NICHD |
| | P. Kane, Biologist Tech | LMG:NICHD |
| | S. Shin, Summer IRTA | LMG:NICHD |
| | A. Ma, Summer IRTA | LMG:NICHD |

COOPERATING UNITS *(if any)* H.-J. Thiesen, Basel Inst. Immunol., Basel, Switzerland; P. Lichter and D. Ward, Yale University, New Haven, CT; B. Brooks and R. Feldman, DCRT, NIH; W. Egan and R.A. Byrd, FDA/CEBR.

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Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.6 | 1.8 | 0.8 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nucleic acid binding proteins are of major importance in biology since they include many of the factors that regulate gene expression and developmental processes. One major class of such factors are constituted by the family of zinc finger proteins, defined by conserved structural motifs which require zinc ions for appropriate folding into active forms. This project is focused on a genetic and molecular analysis of zinc finger proteins and their genes in different biological systems. (1) How many zinc finger proteins are encoded in the genome and how are these genes distributed on the chromosomes? This question is being asked in the human with regard to the C2H2 family of zinc finger proteins. Over 200 genomic clones have been isolated and are being sorted into groups of overlapping sequences that represent distinct loci, providing evidence for at least 20 finger loci in the human genome. Most of these loci have been mapped onto the human chromosome complement by in situ hybridization. By extrapolation to additional clones obtained in this and in other laboratories it can be estimated that the human genome contains at least one hundred, and possibly several hundred, genes for zinc finger proteins of the C2H2 class. (2) What is the structure and mechanism of interaction of the C2H2 finger domains with nucleic acids? This question is being addressed through a detailed structural analysis of the finger regions from several known nucleic acid binding proteins. A database of Zinc Finger proteins has been established for the purpose of statistical and structural modeling of the individual finger regions. Using constrained molecular dynamics (MD) to analyze the structural potentials for the finger domains from Xenopus laevis TFIIIa, mouse mkr2, and yeast Adr1, we have identified two general structural motifs. Fingers with primary structures that fit the consensus homology may fold to form short stable $\alpha$ to 3,10 helical regions on the histidine side of the domain, while nonconsensus fingers appear to have a more extended $\beta$ loop structure. Potential energy analysis from the MD simulations suggested that fingers 1 and 3 have the least and most stable structures, respectively, among TFIIIA finger domains. This structural instability has been confirmed through 1D $^1$H NMR spectroscopy of chemically synthesized TFIIIA finger 1.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 01008-01 LMG

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Genetics of Protein-Nucleic Acid Interactions in Drosophila

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | S. Haynes | Senior Staff Fellow | LMG, NICHD |
| Others: | P. Banerjee | Student Volunteer | LMG, NICHD |

COOPERATING UNITS *(if any)*
Ann Beyer, Department of Microbiology, University of Virginia Medical School

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Laboratory of Molecular Genetics

SECTION
Section on Developmental Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A family of genes encoding putative RNA binding proteins has been identified in Drosophila. Three members of the family have been characterized, two in detail. The two well-characterized genes, Hrb98DE and Hrb87F, encode the major protein components of nuclear ribonucleoprotein complexes. These proteins are closely related in sequence and structure to each other and to the A and B group proteins of vertebrate hnRNP complexes. Both genes show a high level of expression maternally, reduced but significant expression during late larval and pupal development, and low levels of expression during the rest of development. In situ hybridization of gene-specific probes to developing embryos has shown that the early maternal expression is spatially uniform but later expression is localized predominantly to the nervous system and a few other tissues. Antisera produced against a fusion protein reacts with both the Hrb98DE and Hrb87F proteins and identifies >12 spots on two-dimensional immunoblots. Antisera specific for individual proteins have allowed the identification of many of the spots. The proteins migrate as an ~50S ribonucleoprotein complex in sedimentation analysis, and the integrity of the complex is dependent on the integrity of the associated RNA, as expected for hnRNP proteins.

The third gene, identified by the R16 cDNA clone, is more distantly related to the Hrb genes than they are to each other. Like the Hrb proteins, the N-terminal half of the R16 protein has two copies of the RNP motif, a conserved 80 amino acid motif found in many RNA binding proteins. The C-terminal half is distinct, and encodes a proline-rich domain. Northern blot analysis indicates that at least three transcripts are expressed, and their levels vary during development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00801-15 DPR

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies Based on the Medical Birth Registries of Norway and Sweden

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H.J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |
| Other: | A.A. Herman | Visiting Scientist | EB, DPR, NICHD |

COOPERATING UNITS *(if any)* Inst. of Hygiene & Soc. Med. & Dept. of OB/GYN, Univ. of Bergen, Norway (P. Bergsjø & L. Irgens); Dept. of Community Medicine, Univ. of Trondheim and Nat'l Inst. of Public Health, Oslo, Norway (L. Bakketeig, A. Arntzen); Dept. of OB/GYN and Social Med., Uppsala Univ. (G. Lindmark, S. Cnagtingius, O. Meirik).

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NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .1 | .1 | .0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

These studies have focused on: (1) the relation of the quality of medical care to the risk of perinatal death in Norway and Sweden, (2) the tendency to repeat similar birth weight and gestational age in subsequent pregnancy outcomes to the same mothers, (3) perinatal mortality in relation to order of birth and size of sibship, (4) epidemiologic risk factors for preterm birth, (5) epidemiologic risk factors for small-for-gestational age births, (6) contribution of multiple births to perinatal mortality rates, (7) size at birth as measured by head circumference, crown-heel length, and birth weight in relation to gestational age and perinatal mortality.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00802-15 DPR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Linked Live Births-Infant Deaths and Fetal Deaths from U.S. States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |
| Others: | M. H. Malloy | Research Medical Officer | EB, DPR, NICHD |
| | A. A. Herman | Visiting Scientist | EB, DPR, NICHD |
| | M. D. Overpeck | Health Statistician | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*
Departments of Health in the following states: Michigan, Missouri, New York State, North Carolina, and Utah; Office of International Statistics, NCHS (R. Hartford).

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Office of the Director, DPR

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INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .2 | .2 | .0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives are to assemble a multi-state data file of infant deaths in which prior linkage with birth certificate information has been performed. Similar information regarding fetal deaths, based on reports filed for fetuses of at least 20 weeks gestation, will also be studied. The studies to be done on the data set include associations between infant and fetal mortality with the standard information on birth certificates (e.g., birth weight, gestational age, maternal age, race, parity, etc.). The information on fetal or infant death records includes immediate and underlying cause-of-death categories corresponding to the International Classification of Diseases (ICD), based on either the eighth or ninth revision of the ICD codes. Some additional data are available from selected states regarding: smoking during pregnancy, maternal prepregnant weight and height, weight-gain during pregnancy, occupation of parents, and the levels of obstetric and pediatric care available to mother and infant.

Several research contracts have been jointly funded by NICHD and NIEHS to provide data from selected U.S. States (listed above) to compare with data from other developed countries (Australia, Japan, Norway and Scotland) for the time period, 1980-84. This study is entitled: Multinational Comparisons of Birth Weight-Specific Perinatal Mortality Rates.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00803-06 DPR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Sudden Infant Death Syndrome (SIDS) Risk Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |
| Other: | H. H. Song | Guest Researcher | BMSB, DPR, NICHD |

COOPERATING UNITS *(if any)* U. of MD (D. Denman); U. Wash., (D. Peterson, G. van Belle); Loyola U. (J. Goldberg); UCLA (R. Harper, J. Kraus); Columbia U. (J. Parker, E. Krongrad); N.Y. State Hlth Dept. (S. Standfast); U. Mo. (L. Hillman); U. London, U.K. (D. Southall); U. Miami (M. Dapena); U. NM (P. McFeeley); AFIP (T. Stocker).

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INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .3 | .2 | .1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NICHD SIDS Cooperative Epidemiological Study was designed to enable identification of risk factors which could differentiate SIDS infants from non-SIDS infants. The design is that of a multicenter, population-based, case-control study with a sample of 838 SIDS cases (800 singleton and 38 multiple birth SIDS cases) ascertained under a common necropsy protocol. There were 1,600 matched living singleton control infants and 40 co-multiple birth control infants recruited into the study. It is the largest detailed epidemiological study of SIDS ever undertaken. Data were collected for babies who died over a 15-month period from October, 1978 through December, 1979. Every infant death was autopsied in accordance with a common necropsy protocol developed specifically for the study. Twenty-six different slides of tissues were preserved for detailed examination by a panel of three SIDS pathology experts. Under an Inter Agency Agreement with the Armed Forces Institute of Pathology (AFIP), technical support is being provided for the preparation of a SIDS Histopathology Atlas and "study sets" to be used for the education of practicing forensic pathologists or pathology students. Also, the possible association between elevated fetal hemoglobin (Hb F) levels and SIDS is being investigated in a study begun during the past year.

In another SIDS risk factor study, techniques of time series analysis are being used to examine potential abnormalities in the development of neuro-physiological and cardio-respiratory control mechanisms in the first three months of life. The study materials consist of computerized data sets from long-term electrophysiological recordings of infants from three earlier SIDS research studies. Comparisons have been made among the following groups of infants: subsequent siblings of SIDS infants, "near-miss" infants, twins, matched controls, and infants who later died of SIDS.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00841-09 DPR |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Methods for Comparing and Analyzing Data from Several Complex Surveys

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |

COOPERATING UNITS *(if any)*
BB, CTS, DCPC, NCI (B. Graubard); Biomathematics Department, School of Medicine, (E. Korn).

LAB/BRANCH
Office of the Director, DPR

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INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS:<br>.2 | PROFESSIONAL:<br>.2 | OTHER:<br>.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study will develop statistical methods for the analysis of data from complex sample surveys and test them empirically using the National Health and Nutrition Examination Survey I and II (NHANES). The research concentrates on ways to test hypotheses about the coefficients that derive from multiple linear regression of survey data. Existing methods which use Wald test statistics are compared to newly developed approaches based upon Bonferroni t-statistics and jackknifed Wald statistics. These regression methods will be applied to the NHANES data sets to determine if they can be used to provide ways to analyze the complex relationships of growth and nutrition.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00860-10 DPR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Biomedical Time Series Data

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. J. Hoffman | Special Ass't for Infant Mortality | DPR, NICHD |
| Other: | K. F. Yu | Mathematical Statistician | BMSB, DPR, NICHD |

COOPERATING UNITS *(if any)* CI, CP, GRC, NIA (M. Brock); Dept. of Pediatrics, Univ. of S. Florida College of Medicine, St. Petersburg, Florida (B. Bercu); Pediatric Nutrition, Mead Johnson Company (J. Hansen); Univ. of Cambridge, England (K. Dalton and G. Breborowicz).

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SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .1 | .1 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are: (1) to characterize developmental patterns from daily measurements of gonadotropins and for estrogens in premenarchial girls and pubescent boys based on radioimmunoassay methods for measuring urinary luteinizing hormone, urinary follicle stimulating hormone, and urinary estradiol, estriol and estrone hormones; (2) gonadotropins in both castrated and intact male monkeys of different ages; (3) growth hormone in normal and precocious pubertal children; (4) to assess circadian and other rhythms in heart rate, temperature and other serial data collected from long-term studies in humans; and (5) to perform analysis of these serial measurements using methods of statistical time series analysis, including autoregressive filtering, auto- and cross-spectrum analysis, and robust smoothing procedures.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00861-08 DPR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of In-Utero Fetal Growth Patterns in Relation to Outcome at Birth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |
| Other: | H. Quan | Visiting Fellow | BMSB, DPR, NICHD |

COOPERATING UNITS *(if any)* D. Denman (Univ. of MD); CRMC, NICHD (D. McNellis); Univ. of Trondheim, Norway (G. Jacobsen, L. Bakketeig); U. of Bergen Norway (P. Bergsjø, T. Evans, T. Markestad); Uppsala Univ., Sweden (G. Lindmark); Vanderbilt U., TN (G.W. Reed); U. of AL in Birmingham (R. Goldenberg, K. Nelson).

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NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .2 | .2 | .0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project has been expanded to encompass two related research studies. The first study has analyzed data derived from a randomized clinical trial of diagnostic ultrasound use during pregnancy conducted by the team of Norwegian investigators in Trondheim, Norway. The purpose of the analysis is to examine fetal growth patterns using longitudinal measurements throughout pregnancy of: (1) symphyseal-fundal heights; (2) weight gain at each prenatal visit; (3) serial biparietal and abdominal diameter measurements from ultrasound; and (4) maternal hemoglobin level. Regression models have been fit to the serial measurements for each mother. The coefficients of the regressions have been analyzed in relation to various indicators of birth size such as weight, crown-heel length, ponderal index, and birth weight-for-gestational age percentile. Using an analysis of covariance procedure, additional factors (e.g., cigarette smoking, alcohol intake, low maternal prepregnancy weight, etc.) will be tested for significance in modifying intrauterine growth patterns.

In addition to the study described above, a prospective study to determine risk factors for intrauterine growth retardation, or small-for-gestational age birth, was begun in 1984 through the research contract mechanism with both the University of Alabama in Birmingham and University of Trondheim, Norway (in collaboration with the Universities of Bergen and Uppsala). The study protocol includes recruitment of pregnant women before 17 weeks gestation. Those enrolled in the study will be carefully monitored throughout the remainder of their pregnancy. Symmetric and asymmetric forms of intrauterine growth retardation will be assessed prenatally and at delivery. Infants born to the study mothers will have follow-up exams during the first year of life to assess catch-up growth and attainment of early developmental milestones.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00871-05 DPR |

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Trial of New Drug Therapy for Cystinosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |

COOPERATING UNITS *(if any)* BS, EBB, DMID, NIAID (G. Reed); HGB, IRP, NICHD (W. Gahl); Univ. California, San Diego (J.Schneider); Univ. of Michigan Medical School (J. Thoene); Univ. of Texas Health Science Center, Dallas (J. Reisch); NIAID (G.F. Reed).

LAB/BRANCH

Office of the Director, DPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .2 | .2 | .0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Cysteamine Study provided answers to the question of the drug's efficacy with some inferential difficulty, since cysteamine's unpleasant taste and smell rendered it unpalatable to many patients, who subsequently did not receive effective amounts of the drug. The design of the study itself, with no randomized concurrent control group, obscured effects and required a good deal of reliance on adjustment techniques in the final analysis.

The object of the current study is to improve treatment of cystinosis and determine more of the effects of cysteamine. In the drug development phase of the trial, investigation of a cysteamine analog, phosphocysteamine, revealed that it converts rapidly to cysteamine in the bloodstream, so that the two drugs are effective equivalents. Moreover, since the taste and smell of phosphocysteamine are less obnoxious to some patients, it serves as an alternative treatment that may improve patient compliance. The current study randomizes patients to a low dose of cysteamine (or phosphocysteamine as the patient chooses) or to a high dose; so designed the trial is an optimal vehicle for ascertaining the best course of treatment.

Patient recruitment and treatment is coordinated at contracted study center at the University of California, San Diego. Data center functions are the responsibility of the University of Texas Health Science Center at Dallas. The study will encompass 3-4 years of enrollment and treatment of at least 90 patients. The treatments will be evaluated on the basis of renal function as measured by serum creatinine levels and creatinine clearance, as a surrogate of glomerular filtration rate, at the end of the study.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00872-05 DPR |

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Factors Associated with Premature Births: Missouri Follow-back Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.J. Hoffman Special Ass't for Infant Mortality OD, DPR, NICHD

COOPERATING UNITS *(if any)*

Missouri Department of Health (G. Land, W. Schramm, J. Stockbauer, & V. Pierson).

LAB/BRANCH

Office of the Director, DPR

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INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .2 | .2 | 0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective is to obtain more accurate information relating to the very low birth weight (VLBW) infant, <1500 grams, than is now available from the United States vital records. This objective will be accomplished by the following: (1) mailing or administering a questionnaire to mothers of VLBW infants, mothers of fetal deaths, and a sample of mothers of LBW infants (1,500-2,499 grams) and normal birth weight infants (≥2,500 grams) in order to obtain and verify information from the prenatal, perinatal, and post-neonatal periods; (2) conducting telephone follow-up interviews on non-respondents and incomplete respondents, and a 10 percent sample of study mothers to obtain and/or verify information on the questionnaires; (3) developing procedures for abstracting information from hospital and physician records, including otherwise unavailable or missing information on morbidity, lifestyle, and socioeconomic indicators of the study subjects; and (4) preparing an edited data tape for NICHD. In addition, mortality and results of follow-up evaluations will be available through the first year of life for this birth cohort.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00875-02 DPR |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
National Maternal and Infant Health Survey (NMIHS)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |
| Other: | D. A. Bryla | Statistician | BMSB, DPR, NICHD |

COOPERATING UNITS *(if any)*
Office of Planning and Evaluation, NICHD (G. Gaines); Followback Survey Branch, National Center for Health Statistics (P. Placek, K. Keppel, G. Simpson, M. Kogan).

LAB/BRANCH
Office of the Director, DPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .1 | .1 | .0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A National Maternal and Infant Health Survey is being conducted by the National Center for Health Statistics with the joint sponsorship of NICHD and several other Public Health Service and federal agencies. The survey consists of three components: a natality survey, which is sampled from certificates of live birth; a fetal mortality survey, which is sampled from reports of fetal death, and an infant mortality survey, which is sampled from infant death certificates. In the summer of 1987, a four-State pretest was conducted for 200 live births, 200 fetal deaths, and 200 infant deaths. Questionnaires were mailed to mothers, hospitals, and prenatal care providers associated with these events. Sponsor agency questions were included in the pretest and then revised as needed for the main survey. Final OMB approval was obtained in the fall of 1988, and the main survey was launched in January 1989. NCHS is monitoring data collection which is being done by the Census Bureau.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00323-10 EB |
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PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
District of Columbia Perinatal Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H.W. Berendes | Director | DPR, NICHD |

COOPERATING UNITS *(if any)*
Epidemiology Branch, DPR, NICHD (L.C.Cooper)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.4 | 0.1 |

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☑ (a1) Minors
☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The D.C. Perinatal Study is a case-control study designed to elucidate the factors associated with the delivery of a low birth weight infant to resident mothers in the District of Columbia. The study "cases" were low birth weight infants (<2500 grams) born in participating hospitals. "Controls" were selected as the next race matched normal weight infant (= >2500 grams) delivered at the same hospital. The mothers of the cases and controls were interviewed on the postpartum ward, with data verification obtained through abstraction of medical records. Where possible, prenatal information was verified by using the prenatal information which was attached to the hospital medical record. However, if the hospital medical record did not contain adequate prenatal information arrangements were made to abstract this information from private and public physician's offices where care was received. Data collection began February 1, 1984, and continued until January 31, 1985. The data was collected by SRA Technologies, Inc., of Arlington, Virginia.

In September 1985 SRA returned the data instruments to NICHD due to an inability to complete the contract. Raw data was returned as well as data entered on two data tapes and disk through the Division of Computer Research and Technology (DCRT). It was necessary to re-key all of the data originally submitted by SRA Technologies. One hundred percent of the data have now been keyed. Manuscripts are now in the process of being prepared for submission to peer reviewed journals. Three abstracts were presented at the American Public Health Associations 116th Annual meeting in Boston, Massachusetts, November 13-17, 1988. A number of other speaking engagements have followed or been scheduled. Other speaking engagement on the DC Perinatal Study include: Copin State College, School of Nursing, Sept. 1989; Howard University, School of Community Denistry, Oct. 1989; Black Nurses Association's Annual Meeting, Nov. 1989; DC Health Department Advisory Group, June 1989.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00325-09 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neural Tube Defects and Folate

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.L. Mills Research Medical Officer EB, DPR, NICHD

COOPERATING UNITS *(if any)*

Office of the Director, DPR, NICHD (H.Hoffman)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS:<br>0.2 | PROFESSIONAL:<br>0.2 | OTHER:<br>0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

  ☑ (a1) Minors

  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Epidemiology Branch (DPR) has completed a case-control study of neural tube defects in Illinois and California. Two major reports have come out of the study. Additional analysis is taking place to look at the descriptive epidemiology of neural tube defects and to explore genetic factors in the etiology of neural tube defects.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00329-08 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of an Intervention Trial to Prevent Low Birth Weight in D.C.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H.W. Berendes | Director | DPR, NICHD |
| Other: | M.D. Overpeck | Health Statistician | EB, DPR, NICHD |
| | A.A. Herman | Visiting Scientist | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

Greater Washington Research Center, Washington, DC (J.Maxwell); Better Babies Project, Washington, DC (D.Coates).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.3 | 0.2 | 0.1 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

  ☑ (a1) Minors

  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Better Babies Project (BBP) pilot study was a three-year research and demonstration effort funded by private foundations to reduce the rate of low birth weight and associated infant mortality and illness in a specific high risk area of the District of Columbia. The BBP Service Delivery team began collecting data July, 1984, for the project's mini pilot. As a result of the mini pilot findings a number of revisions were made in the forms and interventions. These revised forms and interventions were then developed and piloted. A four year trial of the project began September, 1986 and ended in 1990. The Project has attempted to identify all pregnant women in a high risk area, help link them with existing medical, social, and health services, facilitate their use of these services, and provide health education and social services.

NICHD had funded two contracts for the BBP to assist with the evaluation. Data management and analysis is currently being done by Computer Data Systems, Inc. The D.C. Department of Human Services, Research and Statistics Division, through a contract with NICHD, is providing us information on all pregnant women delivering in the District of Columbia during the period of the project. Analyses of preliminary data should be completed by June 1991.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00331-07 EB |

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diabetes In Early Pregnancy Project (DIEP)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J.L. Mills | Research Medical Officer | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

Cornell Univ.Med.Center, NY (L.Jovanovic); Brigham and Womens Hosp. Boston, MA (L.Holmes); Northwestern Univ.Med.Center, Chicago, IL (J.L.Simpson); Univ.of Pittsburgh, Pittsburgh, PA (J.Aarons); Univ. of Washington, Seattle,WA (R.Knopp).

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.8 | 0.8 | 0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☑ (a1) Minors
- ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The number of issues relating to diabetic control, growth in pregnancy, and malformations are currently being addressed. An analysis of glycose control in relation to macrosomia has been completed and tentatively accepted for publication at the American Journal of Obstetrics and Gynecology. An analysis of early growth in diabetic and normal pregnancy as measured by ultrasound has been completed and is submitted for publication. An analysis of cholesterol, triglyceride and blood pressure changes in early diabetic pregnancy has been completed and is being submitted for publication. Analyses are underway in the areas of patterns of minor malformations in the DIEP, HLA types as a factor in congenital malformations and ketone patterns in early diabetic pregnancy. Blood specimens have been shipped to Sansum Medical Research Foundation to be analyzed for glycosylated proteins.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00334-07 EB |

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Low Birth Weight Across Generations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.A. Klebanoff | Research Medical Officer | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

Office of the Director, DPR, NICHD (H.W.Berendes); World Health Organization, Geneva, Switzerland (O.Meirik); University of Pennsylvania (S.Katz), University of Southern California (B.Mednick)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .65 | .65 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The original description of the association of maternal and infant birth weights was followed by the description of the association between large maternal birth weight and delivery of a macrosomic (>4000 gram) infant. Study of other fetal growth parameters, including length and head circumference, demonstrated that infants of low birth weight mothers were both shorter and lighter than infants of larger mothers, but that the infants were normally proportioned.

In related studies, birth certificates of infants born in Tennessee between 1979 to 1984 were matched with those of their mothers, who were born in Tennessee between 1959 to 1966. Maternal and infant birth weights were again shown to be correlated. In addition, women who were themselves of low birth weight were up to 4 times as likely to have a small for gestational age infant as were women weighing 4000-4499 grams, but the low birth weight women were less than twice as likely to have a preterm infant. A group of Swedish women, born from 1955 to 1965, was studied. Women themselves smaller gestational age at birth were at increased risk of giving birth to both small for gestational age and preterm infants. Women who were preterm at birth were not at increased risk of either outcome.

Follow-up of girls who were born in the 1960's as subjects in the Collaborative Perinatal Project and Danish Perinatal Study is currently underway in order to examine their reproductive histories. Small for gestational age, preterm and control girls have been located and interviewed. Hospital records of their confinements have also been retrieved.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00340-07 EB |

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ethnic Differences in Birth Weight and Length of Gestation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.C. Cooper Nurse Epidemiologist EB, DPR, NICHD

COOPERATING UNITS *(if any)*

Office of the Director, DPR, NICHD (H.W. Berendes)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .3 | .3 | 0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A contract to obtain a quantifiable description of behavior and lifestyle differences among pregnant women of different ethnic groups which are known to differ in their rates of low birth weight has been awarded to Columbia University and to Northwestern University. The overall goal of this project is to define previously undescribed risk factors affecting birth outcome from pregnant women in the following ethnic groups: American Blacks, Chinese, Mexican-Americans, Puerto Ricans, Dominicans, and Whites. The work scope of the contract includes development of an extensive questionnaire by a multidisciplinary team of experts, pretesting of the interview instruments, interviewing pregnant women from the six groups noted above, and preparing an edited data tape of all responses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00344-07 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Long Term Health Effects of Infant Formulas Deficient in Chloride

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.H. Malloy Research Medical Officer EB, DPR, NICHD

COOPERATING UNITS *(if any)*

Office of the Director, DPR (H.Berendes), DCPC, NCI (B.I. Graubard), CRMC, NICHD (A.Willoughby)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

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|---|---|---|
| TOTAL MAN-YEARS:<br>0.7 | PROFESSIONAL:<br>0.5 | OTHER:<br>0.2 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☑ (a1) Minors
- ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During 1978 and 1979 two infant formulas deficient in chloride were marketed in the United States. It has been estimated that a minimum of 20,000 infant years of these formulas were purchased and more than 100 children were reported to the Centers for Disease Control with metabolic and other abnormalities, principally hypochloremic metabolic alkalosis. In a study of 21 of these children at 2 years of age a significant inverse correlation between length of exclusive use of defective formula and cognitive outcome as measured by the Bayley Scales of Infant Development (r=.55, p=.01) was noted. In a population-based study which ascertained the infant formulas used by first and second graders attending public school those who were exposed to defective formula scored lower on the general cognitive index and the quantitative scale (McCarthy) than did the children who used other soy formulas.

To substantiate these findings a further study of children was carried out in the metropolitan Washington, DC, area schools. 188 children exposed to deficient formula and 479 matched control children exposed to other soy formulas have been tested. In addition, approximately 39 children with a documented history of hypochloremic metabolic alkalosis resulting from defective formula use were brought to the Washington area for testing. The performance of all these children on a battery of psychological tests have been measured and a careful statistical analysis has been completed to look for an effect of exposure to the defective formula with and without documented illness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00346-06 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Time Trends in the Incidence of Biliary Atresia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Klebanoff Research Medical Officer EB, DPR, NICHD

COOPERATING UNITS *(if any)*

Case Western Reserve University (B.Chatterjee)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 0.02 | PROFESSIONAL: 0.02 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Extrahepatic biliary atresia is a liver disease presenting in early infancy, manifested by progressive obliteration of the extrahepatic bile ducts. It has been estimated to occur in from one per 8000 to one per 15000 live births, and is the single most common indication for performance of liver transplantation in children. None of the incidence figures is based on a well defined geopolitical region; most estimates of the frequency of this condition are derived from referral centers. Some investigators have suggested a time-space clustering of this condition.

This project has gathered birth certificates and other information on all cases occurring among children born over a period of 12 years in Ohio. Ninety-four (94) cases were identified, corresponding to a rate of 0.5 cases/10,000 births. Cases will be compared to the other births in the state for evidence of changes in incidence and clustering. A number of potential risk factors for the condition will be examined.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00352-05 EB |

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Human Immunodeficiency Virus - Related Problems

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.H. Malloy Research Medical Officerh EB, DPR, NICHD

COOPERATING UNITS *(if any)*
Office of the Director, DPR (H.W.Berendes), HRSA (S.S.Kessel), American Academy of Pediatrics (C.Croft, G.Fleming).

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

|  |  |  |
|---|---|---|
| TOTAL MAN-YEARS: 0.5 | PROFESSIONAL: 0.5 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☑ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

NICHD/DPR is involved in helping the American Academy of Pediatrics (AAP) develop an education program for pediatricians that deals with developmental sexuality and AIDS. The Academy has designed a program that calls for the development of an educational package by a group of experts in human sexuality, adolescent medicine and human development. The educational package is then to be administered to a randomly selected group of pediatricians. Follow-up of the pediatricians who receive the education program and follow-up of a group of pediatricians who did not receive the protocol will be carried out to determine if the program affected the pediatricians' behavior in the office setting. To date the program has been administered to approximately 100 pediatricians. The pediatricians received the program well and the evaluation component is underway.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 00360-04 EB

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Prospective Study of 1st Trimester Use of Bendectin and Malformations

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.A. Klebanoff | Research Medical Officer | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*
Packard Foundation, Los Altos, CA (P.Shiono)

LAB/BRANCH
Epidemiology Branch

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NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: .05 | PROFESSIONAL: .05 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Most previous studies on this topic used a retrospective case-control design or indirect measures of exposure (pharmacy records). In this prospective study, 31,602 women were asked at their first prenatal visit about the use of Bendectin; 2,711 women reported use in the first trimester. The odds ratio (and 95% interval estimates) for major malformations was 1.05 (0.78-1.40). When individual malformations were evaluated, Bendectin use was statistically associated with microcephaly (5.33 (1.61-17.7)), cataract (5.33 (0.98-29.1)), and lung malformations (4.58 (1.76-11.9)). Since it is not clear whether these associations are due to the use of Bendectin or to the indication (vomiting) for which the drug was prescribed, the association between vomiting and these malformations was studied using previously published data from the Collaborative Perinatal Project. In that study, vomiting was associated with microcephaly (3.3 (1.1, 9.8)) and cataract (3.5 (0.8-16.1)). Vomiting was associated with these two malformations only among nonusers of Bendectin. Lung malformations were not associated with vomiting during pregnancy (1.3 (0.8-2.1)). These data strongly suggest that Bendectin is not associated with these malformations, however the possibility that vomiting is associated with microcephaly and cataract is suggested.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 00361-04 EB

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Child Health Supplement to the 1988 National Health Interview Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.D. Overpeck Health Statistician EB, DPR, NICHD

COOPERATING UNITS *(if any)*
HLB, CRMC, NICHD (P.C.Scheidt); DBSB, CPR, NICHD (V.S.Cain, W.Baldwin); National Center for Health Statistics; Bureau of the Census; Maternal and Child Health, HRSA, Child Trends, Inc. (N.Zill); Centers for Disease Control (Y.Harel)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 0.7 | PROFESSIONAL: 0.7 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This survey provides data on a nationwide representative sample of 17,200 children. Subjects include child care, family relationships, accidents, injuries, poisonings, other childhood morbidity, perinatal events, use of health services, school performance and behavior. It establishes current normative ranges for the U.S. It will provide data for analysis of trends in the U.S. using the 1981 Child Health Supplement for comparisons. The survey was conducted by the U.S. Census Bureau for the National Center for Health Statistics during the 1988 calendar year. Final data have been released. Data analysis is being conducted.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00362-04 EB

PERIOD COVERED

October 1, 1989 through April 15, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nutritional Aspects of Perinatal Epidemiology in Central America

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | José Villar | Expert | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

Office of the Director, DPR, NICHD (H.J. Hoffman)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.4 | 0.8 | 0.6 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

PHS 6040 (Rev. 1/84) GPO 914-918

| | |
|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00363-03 EB |

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NICHD Smoking Trial of Pregnant Women (STOP)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | L.C. Cooper | Nurse Epidemiologist | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*
Office of the Director, DPR, NICHD (H.W.Berendes)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .50 | .50 | 0 |

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The STOP Project will be carried out as a randomized clinical trial to evaluate different approaches to smoking cessation within physician practice settings. This project is a collaborative effort between the NICHD and the American College of Obstetricians and Gynecologists. The unit of randomization in STOP will be a practicing physician's population of pregnant women who smoke or have recently stopped smoking. Physicians will be solicited to volunteer to take part in this randomized study.

There will be two major phases to the STOP Project - a pretest-pilot and a formal trial. The objective of the pilot study is to develop the protocol for the STOP study, develop all study materials (pamphlets, study forms, manual of operations, etc.), recruit private physicians who will assist us in finalizing the study protocol and materials, assist in the development of all quality control procedures, and train the contractor selected to run the formal trial in all aspects of the study. All study materials (forms, urine testing, pamphlets etc.) will be modified to be easily incorporated into the daily routines of private physicians' offices.

The desired result of the pilot will be to have all necessary forms, materials, etc. complete and ready for use in the formal STOP study.

At this time all of our educational materials have been completed and approved by the physicians and staff assisting us in the pretest-pilot phase. On July 27, 1990 we will be holding the scientific peer review for the STOP proposals. It is expected that a contractor will be identified and the contract will be let in October 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00365-03 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Randomized Clinical Trial of Umbilical Artery Catheter Placement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.H. Malloy Research Medical Officer EB, DPR, NICHD

COOPERATING UNITS *(if any)*

Robert Wood Johnson Medical School, UMDNJ (G.G.Rhoads)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 0.25 | PROFESSIONAL: 0.25 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
 ☑ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was formulated to determine if very low birth weight infants who receive umbilical artery catheters that are placed high in the thoracic aorta (T6-T8) are at higher risk of intraventricular hemorrhage than are infants that receive an umbilical artery catheter placed low in the abdominal aorta (L4-L5). We are presently randomizing infants to receive either a high or low catheter and then determining the incidence of intraventricular hemorrhage. The project is scheduled to enroll a total of 700 infants in 12 neonatal intensive care units. A contract for a Data Coordinating Center has been awarded to Scientific Applications Co. To date approximately 500 infants have been enrolled in the protocol. Enrollment should be completed by October.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00366-03 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Survey of Pregnancy Outcomes Among Medical Residents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Klebanoff Research Medical Officer EB, DPR, NICHD

COOPERATING UNITS *(if any)*

Westat, Inc., Rockville, MD; Packard Foundation, Los Altos, CA (P.H.Shiono); Robert Wood Johnson Medical School, UMDNJ (G.G.Rhoads)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .35 | .35 | 0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The effect of stressful employment on pregnancy outcome has been examined in this study of pregnancy outcomes among medical residents. Several studies of paid employment by women during pregnancy have shown an increased risk of both preterm birth and low birth weight associated with strenuous occupations. However, the majority of studies show no increased risk. None of the previous studies were able to control adequately for the socioeconomic status of the women, and in many instances improper controls were used. Women who become pregnant during medical residency are in many respects an optimal group in which to study this issue. They are universally highly educated and in many respects of high socioeconomic status, yet their occupation is highly stressful and physically demanding. For this reason, the effects of a mentally and physically demanding occupation can be studied independently of socioeconomic status. Spouses of male residents comprise an appropriate control group, as they are also of high socioeconomic status, but in most cases have less strenuous occupations than that of a medical resident.

We hypothesize that the mentally and physically strenuous occupation of residency adversely effects the pregnancy outcomes of female residents, as compared to the pregnancy outcomes of spouses of male residents. The proposed study examined pregnancy outcomes among a cohort of 1985 medical school graduates. Approximately 10,000 residents (all of the women residents and a 50% random sample of male residents) were surveyed to determine the pregnancy outcomes of the female residents and spouses of male residents. Women residents were found not to be at increased risk of a variety of adverse pregnancy outcomes compared to spouses of male residents. These results suggest that much of the previously reported occupational influences on pregnancy outcomes were caused by unmeasured differences in socioeconomic status.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00368-03 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Vaginal Delivery of Very Low Birth Weight Infants: Association with Day 1 Deaths

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.H. Malloy Research Medical Officer EB, DPR, NICHD

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.1 | 0.1 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involved the analysis of linked birth and death certificates from Missouri for the years 1980-1984. The 200,000 births occurring during this period are being studied to determine whether or not cesarean sections performed for very low birth weight infants have any advantages over vaginal delivery after adjusting for various complications of delivery. Our preliminary analysis suggests that the vaginal delivery of a very low birth weight infant carries a greater risk of day 1 death than does cesarean section. However, the advantage is lost during the first week of life when those infants delivered by cesarean section have a higher death role than those delivered vaginally. We suspect that the lower first day death rate for infants delivered by cesarean section is related to the greater attention and resuscitative efforts these infants receive. The study has been accepted for publication by the Journal of the American Medical Association.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00369-02 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adverse Perinatal Events and Subsequent Injury-related Death

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.A. Klebanoff | Research Medical Officer | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

ISI, Inc. Alexandria, VA; Epidemiology Branch, NIMH (M. Farmer)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.35 | 0.15 | 0.2 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several published studies have indicated that children experiencing asphyxia during the perinatal period are at increased risk of subsequent adolescent suicide. However, these studies used retrospectively ascertained data and were unable to control for the adverse social conditions that often accompany perinatal difficulties.

In the first phase of this project, the names of the approximately 55,000 children who were born during 1959-66 as subjects in the Collaborative Perinatal Project will be computerized. During the second phase, the computerized names and other appropriate identifying information will be submitted to the National Death Index, and all subjects who have died will be identified. In the third phase, the death certificates of these subjects will be obtained and cause of death recorded. Since the Collaborative Project collected extensive data about the subjects' prenatal, perinatal, and childhood histories, it will be possible to study prospectively the relationship between adverse perinatal events and subsequent risk of death, as well as cause of death.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00370-02 EB |

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ethnic Differences in Hematocrit Levels during Pregnancy and Preterm Delivery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.A. Klebanoff Research Medical Officer EB, DPR, NICHD

COOPERATING UNITS *(if any)*

Division of Research, Permanente Medical Group, Oakland, CA (J.Selby); Biometry Branch, DCPC, NCI (B.Graubard); Packard Foundation, Los Altos, CA (P.Shiono)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .10 | .10 | 0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this study is to assess the role of maternal hematocrit/hemoglobin measures in the occurrence of preterm birth. A recent study by Lieberman et al. (New England Journal of Medicine 1987; 317:343-8) concluded that differences in hematocrit levels between different ethnic groups may account for the well known but otherwise unexplained differences in the risk of preterm birth between the ethnic groups. One phase of this project analyzed prospectively collected hematocrit data from the Collaborative Perinatal Project. The second phase of this project obtained additional data from a subset of women in the Kaiser-Permanente Birth Defects Study to assess the role of maternal hematocrit/ hemoglobin measures in the occurrence of preterm birth.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00371-02 EB

PERIOD COVERED

October 1, 1989 through April 15, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Maternal Dietary Status and Nutritional Status during Pregnancy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.J. Launer Epidemiology Staff Fellow EB, DPR, NICHD

COOPERATING UNITS *(if any)*

Department of Biostatistics, Johns Hopkins University (K-Y. Liang, L. Madger)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 0.75 | PROFESSIONAL: 0.75 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither

  ☐ (a1) Minors

  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

| | |
|---|---|
| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00372-02 EB |

PERIOD COVERED
October 1, 1989 through October 30, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Case-Control Study of the Risk of IVH with High Umbilical Artery Catheters

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: M.H. Malloy Research Medical Officer EB, DPR, NICHD

COOPERATING UNITS *(if any)*

University of Texas Medical Branch (J.Richardson)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.1 | 0.1 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was terminated.

| | |
|---|---|
| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00373-02 EB |

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Calcium Supplementation in Pregnancy to Prevent Preeclampsia and Preterm Birth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J.G. Rigau | Medical Officer III (Epidemiology) | EB, DPR, NICHD |
| Other: | Y.J. Lee | Chief | BMSB, DPR, NICHD |

COOPERATING UNITS *(if any)*

Biometry and Mathematical Statistics Branch, DPR

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.50 | 0.50 | 0 |

CHECK APPROPRIATE BOX(ES)

☑ (a) Human subjects ☑ (b) Human tissues ☐ (c) Neither
- ☑ (a1) Minors
- ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Dietary supplementation with 1.5 g to 2.0 g elemental calcium per day during pregnancy has been found to reduce blood pressure levels and the frequency of preterm birth in two modest randomized trials. It is proposed to test this effect in a larger 2-3 center collaborative, double-masked, randomized clinical trial in which about 4000 women will be enrolled in the first or second trimester and will take 4 calcium tablets (or placebo tablets) daily. Standardized blood pressure measurements will be obtained at entry, at routine prenatal visits and at least once near term. Urinary protein excretion and pregnancy dating will be carefully recorded. Primary outcomes of the trial will be the frequency of pregnancy induced hypertension and of preterm birth. Birth weight will be an important secondary outcome. Proposal reviews have been conducted, and contracts are expected to be awarded in October, 1990.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00374-02 EB |
|---|---|

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Data Coordinating Center for a Study of HIV Infection in Hemophiliacs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | A. Willoughby | Chief | PAMA, CRMC, NICHD |
| Other: | J.G. Rigau | Medical Officer III (Epidemiology) | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*
PAMA, CRMC (A. Willoughby)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.125 | 0.125 | 0 |

CHECK APPROPRIATE BOX(ES)
☑ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☑ (a1) Minors
  ☑ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Multicenter Study of the Natural History of HIV Infection in Hemophilic Children (also titled, for brevity's sake, the Hemophilia Growth and Development Study - HGDS) consists of 1) a network of 14 clinical centers for patient recruitment and a 4-year follow-up, and 2) a data coordinating center for computer and logistical support, forms development, and data entry and limited periodic analyses. The object of the study is to monitor and compare the health of three cohorts of children: HIV-infected hemophiliacs, non-infected hemophiliacs, and non-hemophiliac, non-infected male siblings of hemophiliacs.

For each study participant a conprehensive data base of medical history and health events will be put together, with emphasis on the prospective collection of data on body growth, and endocrine and neuropsychological development. At the conclusion of the second year of operation (May 1988 - April 1990), the data coordination center for HGDS (New England Research Institute, Inc.) has participated in the finalization of the study design, the standardization of protocols and forms for data flow, recruitment, baseline, follow-up and laboratory examinations, and the continued training of clinic personnel who participate in the study. By the end of April, 1990, 263 patients had been enrolled and evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00378-01 EB

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Evaluation of Mortality of Infants Weighing Less than 750 Grams

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.O. Overpeck | Health Statistician | EB, DPR, NICHD |
| Other: | H.J. Hoffman | Office of the Director | DRP, NICHD |

COOPERATING UNITS *(if any)*

NCHS (K.Prager)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.3 | 0.2 | 0.1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The 1983 birth cohort of the National Linked Birth/Infant Death Data Set was analyzed to document the contribution of live born infants weighing less than 750 grams to the U.S. Infant Mortality Rate. Linked birth and death certificates for the total U.S. have not been available since the 1960's in order to assess the mortality experience of these least viable infants. This information is necessary in order to target interventions to reduce the infant mortality rate. The data set represents an intensive effort by NCHS to obtain data from all states and reporting areas. The analysis has been completed and submitted for publication.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00379-01 EB |
|---|---|

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Data Analysis from the Vaginal Infections and Prematurity Study

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.A. Klebanoff | Research Medical Officer | EB, DPR, NICHD |
| Other: | J.C. Carey | IPA | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*
PAMA, CRMC, NICHD (R.Nugent); NIAID (M.F.Cotch); Research Triangle Institute (A.V. Rao); Columbia University (J. Regan)

LAB/BRANCH
Epidemiology Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .25 | .25 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Vaginal Infections and Prematurity (VIP) Study, jointly sponsored by NICHD and NIAID, is a prospective study designed to investigate the relationship between genital tract colonization with various microorganisms and the subsequent development of preterm birth. It also incorporates a clinical trial of erythromycin to prevent preterm birth among women colonized with *U. urealyticum*, *C. trachomatis*, and group B streptococcus. In addition, the VIP collected a wealth of additional data on a variety of factors possibly linked to pregnancy outcome. This project will analyze these factors.

Questions addressed to date include the predictive value of vaginal Gram stain in the identification of group B streptococcus, the descriptive epidemiology of group B streptococcal carriage, and the relationship between reported physical activity and preterm birth.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00380-01 EB |

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

A Population-based Active Information System on Perinatal Drug Abuse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

|  |  |  |  |
|---|---|---|---|
| PI: | A.A. Herman | Visiting Scientist | EB, DPR, NICHD |
| Other: | L.C. Cooper | Nurse Epidemiologist | EB, DPR, NICHD |
|  | J.G. Rigau | Medical Officer III (Epidemiology) | EB, DPR, NICHD |
|  | J.L. Mills | Research Medical Officer | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

|  |  |  |
|---|---|---|
| TOTAL MAN-YEARS:<br>.25 | PROFESSIONAL:<br>.25 | OTHER:<br>0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Epidemiology Branch, DPR, is designing a population-based active information system for perinatal drug abuse. In the first year the project will consist of a conference and a pilot program. The conference will bring together individuals actively involved in the field of perinatal drug abuse. Specifically, we will use this opportunity to examine existing drug information systems, the predictive accuracy of various screening tests, the size of the problem of perinatal drug abuse and availability and efficacy of treatment. The pilot program will be instituted in one or more hospitals or health care centers in a major metropolitan center. The objectives of this pilot program are a) to examine the feasibility of a population-based active information system, b) to examine the predictive accuracy of various screening and diagnostic tests, and c) to investigate the maternal and neonatal health problems associated with perinatal drug abuse.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00381-01 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methods for Analyzing Prenatal Care: Cohort vs Conditional Analysis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.H. Malloy | Research Medical Officer | EB, DPR, NICHD |
| Other: | Y.J. Lee | Chief, BMSB | DPR, NICHD |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.1 | 0.1 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project involves the analysis of Missouri vital statistics data to determine if using a cohort method of analysis is biased in understanding the effect of prenatal care on pregnancy outcome. Cohort methods appear to underestimate the effect of prenatal care and suggest that late entry into prenatal care is more beneficial than early entry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00832-07 EB

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Changes in Perinatal and Infant Mortality by Race in Selected U.S. Cities

PRINCIPAL INVESTIGATOR *(List other professional personnal below tha Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.D. Overpeck | Health Statistician | EB, DPR, NICHD |
| | L.C. Cooper | Nurse Epidemiologist | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

Office of the Director, DPR, NICHD (H.J.Hoffman)

LAB/BRANCH

Epidemiology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 0.1 | PROFESSIONAL: 0.1 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☑ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study describes differences in perinatal mortality and age at death in 59 large cities from 1972 through 1981, during this period of rapid change in technology and medical management of high risk pregnancies.

It explores whether high rates of neonatal mortality in certain cities can be explained by shifts in mortality from the late fetal to the neonatal period and compares differences in perinatal experience according to age at death by race and city size. A secondary analysis of data sets provided by the National Center for Health Statistics was done based on 100 percent reporting of perinatal deaths. Review of fetal death rates from 24 weeks gestational age and of neonatal deaths for the periods, 1-7, and 8-27 days is being used to examine potential reporting differences among cities and shifting of neonatal deaths into the latter period. These data have not been available publicly for analysis. The analysis provides an improved standard for comparison of perinatal mortality in differing geographic sites.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 01307-07 LDMI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pertussis Toxin: An Approach to a New Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J.B. Robbins | Chief | LDMI, NICHD |
| Others: | R. Schneerson | Research Medical Officer | LDMI, NICHD |
| | N.W. Tolson | Biologist | LMDI, NICHD |

COOPERATING UNITS *(if any)*
LCDB, NIDDK (J. Shiloach, B. Kaufman); University of Goteborg, Sweden (T. Lagergard, B. Trollfors, J. Taranger, G. Zackrisson); PRP, NICHD (D.A. Bryla, J.G. Rigau); OD, NICHD (C.U. Lowe); LME, NIDR (J.M. Keith, D.R. Brown).

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.7 | 0.6 | 0.1 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The incidence and severity of pertussis (whooping cough) have been controlled by widespread immunization of infants with inactivated Bordetella pertussis organisms (cellular vaccine or P component of DPT). These vaccines have limitations because they elicit high degree of local and systemic reactions and because they confer immunity for a limited duration of time and only in about 70 to 90% of the population. The identification of pertussis toxin, as a major, if not the sole protective antigen for B pertussis has led to the development of a toxoid derivative of this antigen for vaccine. Pertussis toxin was purified by affinity chromatography from the culture supernatant of B. pertussis and inactivated by a novel method with hydrogen peroxide under controlled conditions. The resulting product, called NICHD-PtXD, has less than 1% of its original binding and enzymatic activities and showed no pharmacologic properties of the holotoxin in in vivo assays such as histamine sensitization. Clinical evaluation in adults, then 18-month old children showed it to be safe and capable of inducing pertussis toxin neutralizing antibodies similar to those observed in children convalescent from disease. Clinical evaluation of NICHD-PtXD in infants used two immunization schedules: three, five and seven months, and three, five and twelve months (recommended schedule for Sweden). The vaccine showed no adverse reactions. In both groups of infants, high levels of antitoxin (neutralized in antibodies) were listed after the third injection. The antitoxin level in the group receiving the toxoid at three, five and twelve months was greater (p = 0.001) than infants injected at three, five and seven months. Clinical effectiveness studies in Sweden are underway.

Covalent binding of pneumococcus-14, a polysaccharide type causing infant infections, to pertussis toxin both inactivated the toxin and induced both antitoxin and high levels of pneumococcus type 14 antibodies in mice.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 01301-08 LDMI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Human Immune Response to Polysaccharide-Protein Conjugate Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | R. Schneerson | Research Medical Officer | LDMI, NICHD |
| Others: | J.B. Robbins | Chief | LDMI, NICHD |
| | A. Fattom | Visiting Associate | LDMI, NICHD |
| | S. Devi | IRTA Fellow | LDMI, NICHD |
| | T. Cramton | Chemist | LDMI, NICHD |
| | D. Pavliakova | Chemist | LDMI, NICHD |

COOPERATING UNITS *(if any)* SUNY Downstate Medical Center, N.Y. (G. Schiffman); Charlotte Memorial Hospital, N.C. (J.C. Parke, Jr.); University of Goteborg, Sweden (T. Lagergard, B. Trollfors, B. Claesson); LCI, NIAID (J. Bennett); LMB, NCI (I. Pastan, D. Fitzgerald); PRP, NICHD (D. Bryla); LID, NIDR (G. Keith, D. Brown); LCMB, NIDDK (J. Shiloach).

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.8 | 2.9 | 0.9 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The age-related and T-independent immunologic properties of Haemophilus influenzae type b capsular polysaccharide and other polysaccharides of invasive bacteria limit their protective actions as vaccines in infants and children, the age group with the highest attack rate of disease due to these pathogens. A general organic synthetic scheme, that could bind H. influenzae type b and other capsular polysaccharides to carrier proteins was devised in order to both increase the immunogenicity of and confer T-cell dependence (booster effect) to these protective antigens. Based upon our, and the work of others, a conjugated Hib vaccine prepared by our original method was licensed by the FDA for universal use in children greater than 18 months of age. Now, a license has been extended to other conjugates and the postmarketing surveillance success of these new products has lowered the age limits to 15 months of age. We have shown that our H. influenzae type b-tetanus toxoid conjugate is both safe and immunogenic in infants at ages 3, 5, 7 and 18 months of age (recommended schedule for infant vaccines). About 75% of the infants developed protective levels of antibody with one injection and all were protected after the third injection. Protective levels of antibodies were induced to the carrier protein as well. This technology has been extended to Streptococcus group B type III, Cryptococcus neoformans and to group B meningococcus capsular polysaccharide which, heretofore, has been considered nonimmunogenic. High levels of antibodies to this polymer were induced by this conjugate in mice. Two lots of penumococcus type 12F-DT conjugate evaluated in adult volunteers were found to be safe and induce higher levels of type 12F antibodies than the unconjugated CP vaccine. The lot made with the higher MW CP induced higher antibody levels. Conjugates of staphylococcal types 5 or 8 and Pseudomonas aeruginosa exotoxin A showed increased immunogenicity and T-cell dependent properties in mice.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 01304-08 LDMI |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protective Effect of Vi and LPS Antibodies Against Salmonellae and Shigellae

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J.B. Robbins | Chief | LDMI, NICHD |

See Attached

COOPERATING UNITS *(if any)*
Pasteur Merieux Serums & Vaccins, France (M. Cadoz); Agency for International Development, Washington, D.C. (F.-Y.C. Lin); OD, NICHD (C.U. Lowe); PRP, NICHD (D.A. Bryla, J.G. Rigau); LCDB, NIDDK (J. Shiloach).

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.8 | 2.8 | 1.0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Enteric bacterial diseases remain a serious and frequent cause of morbidity and mortality throughout the World especially in infants and children. The most serious of these diseases due to Salmonellae and Shigellae. In developing countries, the most frequent and serious of Salmonellosis is typhoid fever caused by Salmonella typhi. The next most common cause varies: In Southeast Asia it is Salmonella paratyphi A and in most of the remaining World it is Salmonella typhimurim (group B). Enteric bacterial diseases due to Shigellae are also serious and frequent. In particular, dysentary caused by these pathogens exerts a profound effect upon future growth and development and has a morbidity and mortality in infants and children. Both may be caused by nontyphoidal Salmonella and Shigella with their peak expression in infancy and young childhood. There is indirect literature to indicate that serum IgG antibodies to the O-specific polysaccharide of the LPS of Salmonellae and Shigellae may confer protective immunity. Accordingly, in order to boost the antibody response, especially the IgG component, to S. typhi, the other Salmonellae and Shigella, conjugate vaccines have been prepared and undergoing laboratory testing. Clinical evaluation of these products is being planned.

| | | | |
|---|---|---|---|
| Others: | R. Schneerson | Research Medical Officer | LDMI, NICHD |
| | C. Chu | Visiting Scientist | LDMI, NICHD |
| | B. Liu | Visiting Scientist | LDMI, NICHD |
| | E.M. Daniels | NRSA Fellow | LDMI, NICHD |
| | T.A. Cramton | Chemist | LDMI, NICHD |
| | N.W. Tolson | Biologist | LDMI, NICHD |
| | J.L. Gal | Adjunct Technician | LDMI, NICHD |

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01310-04 LDMI

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Developmental Gene Regulation of the Immune System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | K. Ozato | Head, SMGI | LDMI, NICHD |

See Attached

COOPERATING UNITS *(if any)*

LCB, NCI (E. Appella); NB, NINCDS (D. McFarlin).

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Molecular Genetics of Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 8.6 | 7.3 | 1.3 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This program addresses regulation of gene expression in the developing immune system. The goal is to elucidate the mechanism of transcriptional regulation of major histocompatibility complex (MHC) class I genes, which play an important role in development and function of the immune system. Our major efforts have been the isolation and characterization of cDNA clones encoding proteins that bind to the conserved cis regulatory elements involved in controlling MHC class I transcription. So far, we have isolated five cDNA clones, three of which have been studied in detail. H-2RIIBP: this is a Zn finger protein that belongs to the nuclear hormone receptor superfamily, and binds to the enhancer region II of the MHC class I gene, and other cis elements, such as the estrogen and thyroid hormone response elements. We show that H2RIIBP trans-activates the MHC class I-CAT reporter gene when transfected into embryonal carcinoma cells. The trans-activation is dependent on the presence of retinoic acid or Vitamin A. RIBP (CRYBP): this protein has two domains of a C2H2 Zn finger. It binds to the enhancer region I, and cis elements of many other genes including the NFkB site in the immunoglobulin, $\beta$-2 microglobulin, and lens crystallin $\alpha$ gene. Monoclonal and polyclonal antibodies raised against RIBP recombinant proteins react with proteins of M.W., smaller than that expected of the cDNA, suggesting that RIBP (CRYBP) undergoes processing. ICSBP: this factor binds to the interferon consensus sequence (ICS), a cis element responsible for interferon (IFN) mediated transcriptional induction of MHC class I gene. The ICSBP belongs to the IRF family and binds to the IFN response elements present in the IFN genes and other genes regulated by IFN. ICSBP is expressed predominantly in lymphoid tissues, and inducible by IFN-$\gamma$, suggesting that it is a multi-functional mediator affecting various aspects of immune regulation. These studies highlight the existence of large families of regulatory genes which have distinct DNA binding domains and are conserved throughout the animal kingdom. Sequencing and initial characterization are still under way for two additional cDNA clones encoding proteins that bind to the negative regulatory element and the enhancer region III of the MHC class I gene. We have also begun analyzing DNA-protein binding in the MHC class I genes in vivo by using the PCR mediated in vivo DNA foot printing procedure.

| | | | |
|---|---|---|---|
| Others: | J.R. Flanagan | Senior Staff Fellow | LDMI, NICHD |
| | S.I. Hirschfeld | Medical Staff Fellow | LDMI, NICHD |
| | A. Dey | Visiting Associate | LDMI, NICHD |
| | P.H. Driggers | Staff Fellow | LDMI, NICHD |
| | M.S. Marks | Staff Fellow | LDMI, NICHD |
| | K.G. Becker | IRTA Fellow | LDMI, NICHD |
| | D.L. Ennist | NRC Fellow | LDMI, NICHD |
| | I.J. Lee | NRC Fellow | LDMI, NICHD |
| | J.H. Segars | AFS Fellow | LDMI, NICHD |
| | T. Nagata | Visiting Fellow | LDMI, NICHD |
| | Y. Kanno | Visiting Fellow | LDMI, NICHD |
| | J. An | Adjunct Scientist | LDMI, NICHD |
| | W.-H. Mak | Chemist | LDMI, NICHD |
| | C. Watson | Bio-Aid | LDMI, NICHD |
| | B. Elenbaas | Summer IRTA | LDMI, NICHD |

| | |
|---|---|
| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 01303-07 LDMI |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Conjugate Induced Polysaccharide Antibodies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | S.C. Szu | Research Chemist | LDMI, NICHD |
| Others: | See Attached | | |

COOPERATING UNITS *(if any)*

University of Missouri-Columbia, Missouri (R. Finkelstein); Tulane University Medical School, Louisiana (J. Clements); IB, NCI (J. Wunderlich).

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Bacterial Disease Pathogenesis and Immunity

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.0 | 4.0 | 2.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Covalent attachment of saccharides, either polysaccharides or oligosaccharides of Gram-negative enteric pathogens to proteins, have been studied for optimum conditions of derivitization, conjugation and presentation as an immunogen to laboratory animals. The properties of the Vi were studied by chemically modifying the prosthetic groups and visualizing the change in structure in a three dimensional model and in bioassays. Conditions were established for removing the O-acetyl and carboxyl groups. The resultant chemically-modified Vi was then studied for its antigenicity and immunogenicity. The O-specific side-chain of Salmonella typhimurium was purified and derivitized by a variety of methods resulting in either site-specific or random activation. The glycolipids of deep rough Gram-negative enteric bacteria, responsible for Gram-negative shock following sepsis in hospitalized patients, were isolated and characterized. These LPS were detoxified with various methods suitable for human vaccines. The O-specific side-chains of V cholera were isolated and several carrier proteins were studied for their ability to form covalent conjugates with these saccharides. Pneumococcal capsular polysaccharides types 23F and 19F were conjugated in organic solvent to prevent decomposition. Conjugates are expected to increase immunogenicity in children and infants.

| Others: | | | |
|---|---|---|---|
| | J.B. Robbins | Chief | LDMI, NICHD |
| | D.C. Watson | Senior Staff Fellow | LDMI, NICHD |
| | R.K. Gupta | Visiting Fellow | LDMI, NICHD |
| | X. Li | Adjunct Scientist | LDMI, NICHD |
| | T.W. Towne | Chemist | LDMI, NICHD |
| | C.K. Gupta | Adjunct Technician | LDMI, NICHD |
| | R.L. Denson | Bio-Aid | LDMI, NICHD |
| | L. Eisiminger | Bio-Aid | LDMI, NICHD |

| | |
|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00073-19 LDMI |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Immune Systems at the Cellular Level

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | E. Hanna | Head, SICC | LDMI, NICHD |

COOPERATING UNITS *(if any)*

LN, NIDDK (P. Arora); OGC, NICHD (M. Walker); VR, DRS (C. Hansen).

LAB/BRANCH
Laboratory of Developmental and Molecular Immunity

SECTION
Section on Immunoregulation and Cellular Control

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland, 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.0 | 0.0 | 0.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

PHS 6040 (Rev. 1/84) GPO 914-918

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00850-14 BB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Randomized, Controlled Study of Phototherapy for Neonatal Hyperbilirubinemia

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. A. Bryla | Statistician | BMSB, DPR, NICHD |
| Others: | H.J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |

COOPERATING UNITS *(if any)* Human Learning and Behavior Branch, CRMC, NICHD (P. Scheidt); Intramural Research, Neuroepidemiology Branch, NINCDS (K. Nelson); Intramural Research, Developmental Neurology Branch (D. Hirtz); Computing Sciences Consultant (K. Fetterly); Biometry Branch, CDTS, NCI (B. Graubard).

LAB/BRANCH

Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .6 | .4 | .2 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study, which began in 1974, is a cooperative, randomized clinical trial to determine the safety and efficacy of phototherapy for treatment of neonatal hyperbilirubinemia by comparing phototherapy with non-phototherapy infants under specific conditions. Babies were randomized by weight (less than 2,000, 2,000 - 2,499 and greater than 2,499 grams) to the phototherapy or non-phototherapy groups. Infants, 2,000 grams and above, were admitted to the study when their bilirubin reached levels specified in the study protocol. All infants under 2,000 grams were admitted. Physical, neurological and mental development of these infants were followed through six years of age.

The Biometry Branch served as a data center for this study and was the focal point for receipt of examination forms. The master files for each year's follow-up were edited for keypunch and coding errors and for internal consistency. The Branch is now analyzing the data in cooperation with the principal investigators from the cooperating units. The results of the newborn data were published in a supplement to Pediatrics in February 1985. One and six-year follow-up data was published in Pediatrics, April, 1990.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00853-06 BB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Design and Analysis of a Clinical Trial of Vi Polysaccharide Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. A. Bryla | Statistician | BMSB, DPR, NICHD |

COOPERATING UNITS *(if any)*

Office of the Director, NICHD (C.Lowe); Laboratory of Developmental & Molecular Immunity, NICHD (J. Robbins); TEKU Hospital, Nepal (I. Acharya).

LAB/BRANCH

Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: .3 | PROFESSIONAL: .3 | OTHER: .0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study is a cooperative, randomized trial to determine the efficacy of Vi polysaccharide in preventing typhoid fever in Nepal. The Biometry & Mathematical Statistics Branch's involvement in this study was to design the data collection forms, and assist in the data management and the analysis with the study investigators from NICHD and Nepal.

In March 1986, 6,908 volunteers from five villages in Nepal were randomly vaccinated with either the Vi polysaccharide or pneumonococcal vaccine. These volunteers were visited every three days for the next two years to verify their health status and to detect any typhoid cases prior to treatment. Blood cultures were done on anyone with a fever of three days duration. Randomization scheme was broken in August, 1988 and cross-immunization of the volunteers took place in September and October, 1988. The data is now being analyzed and a manuscript will be submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00854-06 BB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Analysis of MCH Data from the National Longitudinal Youth Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. A. Bryla | Statistician | BMSB, DPR, NICHD |
| Other: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |

COOPERATING UNITS *(if any)*

Pregnancy and Perinatology Branch, CRMC, NICHD (D. McNellis); Ohio State University (F. Mott).

LAB/BRANCH

Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .1 | .1 | .0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Branch's primary goal in this project is to collect, analyze, and publish data based on a series of annual interviews of young women (aged 14 to 21 on January 1, 1979) regarding their pregnancy outcome and the first year of life of the child. This survey allows analysis of trends over time in the maternal and child health field of, for example, the use of obstetric technology (diagnostic ultrasound, amniocentesis, etc.), and patterns in breast-feeding. This survey has been collecting data on the youth cohort sample in relation to their employment and work history, military service, educational attainments, etc.

The Biometry & Mathematical Statistics Branch (BMSB) began collecting data on pregnancy outcome and the first year of life of the child in 1983. With this six year data base, analysis of trends over time in the maternal and child health will be done.

The BMSB has joined in the funding of the data collection effort together with the Demographic and Behavioral Sciences Branch, Center for Population Research, NICHD. The mechanism of support for the field study is through an Inter Agency Agreement with the Department of Labor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00874-03 BB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Research on Racial Differences in Pediatric Measures of Gestational Age

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Y. J. Lee | Chief | BMSB, DPR, NICHD |
| Others: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |
| | M. A. Klebanoff | Research Medical Officer | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

Research Triangle Institute (V. Rao); NIAID (G.F. Reed).

LAB/BRANCH
Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .2 | .2 | .0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Dubowitz Examination and its derivative Ballard Examination are instruments for estimating gestational age at the time of birth on the basis of observed physical and neurological maturity. Since racial differences in the distribution of some developmental indices are acknowledged or suspected, it is hypothesized that the current pediatric assessments (i.e., the Dubowitz and Ballard tests), which were constructed and validated on a sample of white babies, may effect a bias in the estimation for other racial groups.

The Vaginal Infections in Prematurity (VIP) Study offers data to test the hypothesis. If it is found that differences do exist, the study will also provide the wherewithal to produce a modified pediatric assessment which is proper for the racial group in question. The final data file is expected in late 1990 or early 1991.

| | |
|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00813-08 BB |

PERIOD COVERED

October 1, 1989 to May 31, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biostatistical Methods for the Analysis of Laboratory Research Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | G. F. Reed | Mathematical Statistician | BB, DPR, NICHD |

COOPERATING UNITS *(if any)*

BS, EBB, DMID, NIAID (G. Reed); CPD, CC, NICHD (R. Elin and M Ruddell); IRP, NIAID (D. Alling and S. Banks); Dept. of Statistics, Harvard U. (D Hoaglin); Univ. of MD (D. Denman).

LAB/BRANCH

Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .3 | .2 | .1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is terminated.

| | |
|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00818-08 BB |

PERIOD COVERED
October 1, 1989 to May 31, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Research in Developing Nonparametric Methods for Biomedical Applications

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | G. F. Reed | Mathematical Statistician | BB, DPR, NICHD |
| Other: | H. J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |

COOPERATING UNITS *(if any)*
University of MD (D. Denman); DBSB, CPR, NICHD (B. Graubard).

LAB/BRANCH
Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS:<br>.2 | PROFESSIONAL:<br>.2 | OTHER:<br>.0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is terminated.

| | |
|---|---|
| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00842-08 BB |

PERIOD COVERED
October 1, 1989 to May 31, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of Statistical Methods to Analyze Cluster Samples

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | B.I. Graubard | Mathematical Statistician | DBSB, NICHD |
| Other: | H.J. Hoffman | Special Ass't for Infant Mortality | OD, DPR, NICHD |

COOPERATING UNITS *(if any)*
EMS, BB, EBP, NCI (M. Gail and T. Fears).

LAB/BRANCH
Biometry & Mathematical Statistics Branch

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .2 | .2 | .0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00504-03 LDP

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cloning of the Ah Receptor Gene

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P. I. | Alvaro Puga | NIH Expert | L. Dev. Pharmacology, NICHD |
| Others: | France Carrier | Adjunct Scientist | L. Dev. Pharmacology, NICHD |
| | Anup Dey | Visiting Associate | L. Dev. Pharmacology, NICHD |
| | John E. Jones | Staff Fellow | L. Dev. Pharmacology, NICHD |
| | Lisa A. Neuhold | Biologist | L. Dev. Pharmacology, NICHD |
| | Aprile Pilon-Clayton | IRTA Fellow | L. Dev. Pharmacology, NICHD |

COOPERATING UNITS *(if any)*

Heinrich J. Westphal, Lab. Mammalian Genes and Development, NICHD, NIH.

LAB/BRANCH
Laboratory of Developmental Pharmacology

SECTION
Unit on Microbiology

INSTITUTE AND LOCATION
NICHD. NIH. Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5.34 | 4.42 | 0.92 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the mechanisms that control transcription of the cytochrome P450 Cyp1a1 gene were carried out in three areas: A. Cis-acting regulatory sequences; B. Regulatory pathways; and C. Trans-activating proteins.

A. Cis-acting regulatory sequences. The effect of individual, pairwise and simultaneous mutations of the three distal aromatic hydrocarbon responsive elements (Ah REs) were studied usinf fusions with a reporter gene. In wild type cells, loss of each individual element affected expression to different degrees. The effect was additive in pairwise mutations, and mutations in all three elements practically abolished all expression. In a mutant cell line that lacks P450 activity, basal levels were highly elevated, and addition of inducer had little or no additional effect. These results suggest that the basal expression level is controlled by interactions occurring at the 5'-most AhRE and involving an autoregulatory effect of the cyp1a1 protein.

B. Regulatory pathways. Two other regulatory pathways of Cyp1a1 expression have been uncovered. One consists of the apparent requirement for Ah receptor phosphorylation to bind to its cognate AhRE sequences. The other involves the role that inhibition of hydroxymethylglutaryl coenzyme A reductase plays in derepression of Cyp1a1 transcription. Functional receptor is required, since the effect does not occur in receptorless cells. These data suggest that the Ah receptor may function as a repressor under certain conditions.

C. Trans-activating proteins. A cDNA clone coding for an AhRE-binding protein has been isolated and partially sequenced. Its deduced amino acid sequence contains a possible DNA-binding zinc-finger domain. Its localization to mouse chromosome 2 makes it unlikely that it corresponds to the Ah receptor gene, known to reside on chromosome 12. Several DNA-binding proteins that interact with the AhRE sequences have been isolated by DNA-affinity chromatography. The ligand-binding activity characteristic of the Ah receptor copurifies with these proteins, leading to the conclusion that the Ah receptor is one of them. Antibodies will be used for cloning.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD00136-22 LDP |
|---|---|

PERIOD COVERED
October 1, 1989 to December 1, 1989

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Pharmacogenetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I. | Daniel W. Nebert | Head | L. Develop. Pharmacology, NICHD |
| Others: | Richard Greenawalt | Bio Lab Technician | L. Develop. Pharmacology, NICHD |
| | Ah-Ng Kong | IRTA Fellow | L. Develop. Pharmacology, NICHD |
| | Doron Lancet | Adjunct Scientist | L. Develop. Pharmacology, NICHD |
| | Daniel Petersen | NRC BioTech Fellow | L. Develop. Pharmacology, NICHD |
| | Ming-Huang Zhang | Visiting Fellow | L. Develop. Pharmacology, NICHD |

COOPERATING UNITS *(if any)*

| | |
|---|---|
| A. J. Fornace Jr., | Division of Cancer Treatment, NCI, NIH. |
| F. J. Gonzalez, | Lab. of Molecular Carcinogenesis, NCI, NIH. |
| C. Kozak, | Lab. of Viral Diseases, NIAID, NIH. |

LAB/BRANCH
Laboratory of Developmental Pharmacology

SECTION
Section on Pharmacogenetics and Molecular Teratology

INSTITUTE AND LOCATION
NICHD. NIH. Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.07 | 2.41 | 0.66 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Project terminated

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00343-07 DPR |
|---|---|

PERIOD COVERED
October 1, 1989 through September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Effect of Westernization on Infant Feeding Patterns Among the Negev Bedouins

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H.W. Berendes Director DPR, NICHD

COOPERATING UNITS (*if any*)
Cancer Prevention Branch, NCI, NIH (M.R. Forman); Biometry Branch, DCPC, NCI (B. Graubard); Ben Gurion University of the Negev, Beer Sheva, Israel (L. Naggan)

LAB/BRANCH
Office of the Director, DPR

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| .15 | .1 | .05 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

This is a study of infant feeding practices among Bedouin tribes residing in the Negev, Israel. The objectives are: the evaluation of changes in infant feeding practices during the first year of life and their relationship to physical growth of children and on gastrointestinal and respiratory diseases during the first year of life.

The information obtained covers 5,000 mother-infant pairs. Two samples have been identified; one was identified at birth and a subsample of these births was followed for a period of 5-8 months. Another sample of children was identified at 6 months of age and followed prospectively to 18 months of age.

Several manuscripts based upon the analyses of these data have been published which include: undernutrition among Bedouin Arab infants and effect of infant feeding practices on infant growth, the 40-day rest period and infant feeding practices, and seasonality of births among Bedouin Arabs. The manuscript on perinatal determinants of infant feeding practices has been accepted for publication by the Journal of Pediatric and Perinatal Epidemiology. The manuscript on infant feeding practices as related to morbidity is still in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 01700-03 DPR

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of the Efficacy of IVIG in HIV Infected Children

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H.W. Berendes | Director | DPR, NICHD |
| Others: | A. Willoughby (Co-P.I.) | Head | PAMA, CRMC, NICHD |
| | J. Rigau | Senior Epidemiologist | EB, DPR, NICHD |

COOPERATING UNITS *(if any)*

PAMA, CRMC, NICHD (R. Nugent, L. Mofenson)

LAB/BRANCH

Office of the Director, DPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.1 | 0.1 | 0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The IVIG trial network currently consists of 28 collaborating hospitals. These have enrolled 120 children in group I and 249 children in group II, for a cumulative total of 369 children. Enrollment in group I was closed earlier in the year since the recruitment goal of at least 98 children in group I was reached and also in view of the fact that AZT has now been released for treatment of HIV infection in children by the FDA. Investigators and parents and other care providers of participating children have been advised about the recommendation for the use of AZT in children with HIV infection.

With the merger of NICHD's clinical trials network of hospitals for pediatric HIV trials with the pediatric ACTGs sponsored by NIAID, the administrative direction and coordination of this trial has been shifted from the Office of the Director, DPR to Dr. Willoughby, the Chief of PAMA, CRMC.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01701-02 DPR

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Evaluation of the Impact of a Model Prenatal and Followup Program in Baltimore

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. W. Berendes | Director | DPR, NICHD |
| Other: | A. Herman | Visiting Scientist | DPR, NICHD |

COOPERATING UNITS *(if any)*

Department of Health, Baltimore City (Tom Coyle)

LAB/BRANCH

Office of the Director, DPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 0.2 | PROFESSIONAL: 0.2 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This proposed project is a community based multi-risk factor intervention to improve birth outcome in a geographically defined area of Baltimore. The initiative for this study has come from the Baltimore City Health Department, and DPR has agreed to provide technical assistance, specifically regarding the design of the intervention and the evaluation of the impact of the intervention upon the target population.

Outcome measures to determine the impact of this project include the rate of low birth weight and mean birth weight, as well as change in health habits, especially smoking, drinking and drug abuse, compliance with prenatal care as well as knowledge and attitudes about appropriate health behaviors during pregnancy.

The timetable for the initiation of this project has been shifted since the City Health Department has not been able to secure all the funding from various non-governmental sources as well as some delays in completing the design and protocol for this project. It is now anticipated that this project will be started in September of 1990.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01702-01 DPR

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Infant Feeding Practices Among North African Jews

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | |
|---|---|---|
| PI: H.W. Berendes | Director | DPR, NICHD |

COOPERATING UNITS *(if any)*

Cancer Prevention Branch, NCI, NIH (M.R. Forman); Biometry Branch, DCPC, NCI, NIH (B. Graubard); Ben Gurion University of the Negev, Beer Sheva, Israel (L. Naggan)

LAB/BRANCH

Office of the Director, DPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: .1 | PROFESSIONAL: .1 | OTHER: .0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a study of infant feeding practices among North African Jews who immigrated to Israel during the last decade. The objectives are the evaluation of infant feeding practices and changes during the first year of life in relation to physical growth and morbidity during the first three years of life. In addition it is intended to relate maternal nutritional status during pregnancy to choice and practice of infant feeding during the first few months of life.

The information obtained covers about 1,000 mother-infant pairs. The follow up of these children has been for three years.

The data collection for this project was supported in part by an interagency agreement between the Centers for Disease Control and the WHO.

The data collection is complete and we are planning analysis of the data in the coming year here at DPR.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01703-01 DPR

PERIOD COVERED

October 1, 1989 through September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Study of Pregnancy Outcome, Maternal Death and Child Health in Pakistan

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H. W. Berendes | Director | DPR, NICHD |
| Other: | W. Calvert | Head, Computer Sciences Section | DPR, NICHD |

COOPERATING UNITS *(if any)*

Department of Community Health, Aga Khan University (Drs. John Bryant, Fariyal Fikree, Farid Midhet, and Mehtab Karim), Special Program for Research, Reproduction and Family Planning of the WHO (Dr. Jose Villar)

LAB/BRANCH

Office of the Director, DPR

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.2 | 0.15 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This is a study of maternal and infant mortality in selected sites in Pakistan. The objectives are to develop population based data of the prevalence of maternal and infant mortality in cluster samples of different sites in Pakistan, which include at least one site each in the four provinces of Pakistan and to develop interventions based upon these surveys and evaluate their impact. The study instrument for the assessment of maternal-infant mortality was developed and pretested in Karachi in several Katachi Abadies and covered a total of 10,400 households. Some revisions in the instrument have been made based upon the experience of the pilot study. The next phase is the survey in Baluchistan which will be done in August-September of this year in two sites, including approximately 20,000 households to be followed by Sukkar, Province of Sind in December of this year. Two other sites for this survey in 1991 will be located in the Northwest Territory and in the Punjab.

Another project deals with a cross-sectional longitudinal survey of about 1000-1200 pregnant women in selected Katchi Abadies in Karachi with follow up to delivery and follow up of children to two years of age. The purpose of this project is the identification of major risk factors including maternal nutrition associated with poor birth outcome and infection. Another objective is to differentiate among the low birth weight live birth children from this project preterm birth from intrauterine growth retardation. The follow up of the children is aimed at identifying the role of preterm delivery versus intrauterine growth retardation on morbidity and mortality during the first years of life.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 01606-02 CBMB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Biology of Early Organelles of the Secretory Pathway

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | R.D. Klausner | Head | CBMB, NICHD |
| Others: | J.S. Bonifacino | Visiting Associate | CBMB, NICHD |
| | J. Lippincott-Schwartz | NRSA | CBMB, NICHD |
| | L.C. Yuan | Technician (Chem.) | CBMB, NICHD |
| | C. Suzuki | Adjunct Technician | CBMB, NICHD |
| | J.G. Nuchtern | IRTA Fellow | CBMB, NICHD |
| | Victor Hsu | IRTA Fellow | CBMB, NICHD |
| | Juile Donaldson | NRC BioTech Fellow | CBMB, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Cell Biology and Metabolism Branch

SECTION
Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6 | 5 | 1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This group has been engaged in studying the structure, function and dynamics of the early organelles of the secretory vacuolar system. The group studies three aspects of these problems: 1) Architectural editing - a concept that has evolved out of the work of this group in explaining the cell biologic processes that underlie the ability of the cell to correctly structure newly synthesized membrane proteins and to edit out incomplete or incorrect proteins or protein complexes. One of the major mechanisms by which the cell prevents the expression on the cell surface of abnormal membrane proteins is by targeting those proteins for degradation. This group has identified a new pathway for intracellular degradation that takes place within the endoplasmic reticulum. 2) The structure and dynamics of membrane flow in the secretory pathway - this group has identified a novel drug, Brefeldin A, that has unique effects on organellar structure and dynamics. Based upon the effects of this drug we have characterized new pathways of movement between the Golgi, intermediate compartments, and the endoplasmic reticulum. 3) Antigen presentation - this group has identified the endoplasmic reticulum as the major site for the presentation of endogenous antigens to major histocompatability complex molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01605-04 CBMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The T Cell Antigen Receptor - Structure, Biosynthesis and Cell Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | R.D. Klausner | Head | CBMB, NICHD |
| Others: | D.G. Orloff | Med. Staff Fellow | CBMB, NICHD |
| | S.J. Frank | Med. Staff Fellow | CBMB, NICHD |
| | N. Manolios | Adjunct Scientist | CBMB, NICHD |
| | P. Casson | Adjunct Scientist | CBMB, NICHD |
| | F. Letourneur | Adjunct Scientist | CBMB, NICHD |
| | T. Rutledge | Adjunct Technician | CBMB, NICHD |

COOPERATING UNITS *(if any)*

Biological Response Modifiers Program, NCI (J. Ashwell)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 5 | 5 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goals of this group are to identify the structure and the regulated expression of the T cell antigen receptor complex. In addition, an important component to the work of this group is to utilize mutants combined with recombinant DNA technology to begin to establish structure/function relations for this important immunologic receptor. The structure of two different receptor complexes on the surface of T cells have been defined. Both complexes contain the alpha and beta heterodimeric antigen recognition structures non-covalently coupled to the three membered CD3 complex. The two classes of receptors are distinguished by their containing either a zeta-zeta homodimeric structure or a zeta-eta heterodimeric structure in addition to these five other components. This group has used gene transfection studies to try to address the subunit interactions within the receptor complex. In addition, this group has focused on the zeta and eta chains of the TCR complex, both in terms of the molecular biology, the biochemistry, the assembly and structure/function relations. Eta deficient variants have been isolated and characterized, as well as zeta negative variants and mutants. The eta chain has been biochemically characterized and its immunologic relationship to zeta identified. The zeta chain is transcriptionally regulated in a very intriguing way, and this has been correlated with structural changes in the gene. The full structure of the gene has been identified. The structure of zeta has been altered by site-directed mutagenesis and this has been utilized to reconstruct the first signal transduction mutants of the TCR complex.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01602-06 CBMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Intracellular Iron Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | | | |
|---|---|---|---|---|---|
| PI: | R. D. Klausner | Head | T. Kaptain | Adj. Sci. | CBMB, NICHD |
| | T. A. Rouault | Sr. Staff Fell. | F. Samaniego | Adj. Sci. | CBMB, NICHD |
| | D. Haile | Med. Staff Fel. | | | CBMB, NICHD |
| | A. Dancis | Med. Staff Fel. | | | CBMB, NICHD |
| | J. Barriocanal | Visiting Fel. | | | CBMB, NICHD |
| | J. Harford | Senior Invest. | | | CBMB, NICHD |
| | D. Roman | Clinical Assoc. | | | CBMB, NICHD |

COOPERATING UNITS *(if any)*

American Red Cross, Rockville, MD (W. Burgess)

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 6.5 | PROFESSIONAL: 6.5 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is aimed towards a molecular understanding of the basis of intracellular iron metabolism. A molecular genetic examination of human ferritin has set the basis for defining a cis/trans regulatory model for the post-transcriptional regulation of this critical protein of intracellular iron metabolism. Iron regulates the translation of the mRNA encoding ferritin by virtue of its ability to alter the binding activity of a cytosolic protein that binds to a specific RNA sequence contained within the ferritin message. A complete description of this RNA regulatory element, the RNA binding protein, and how iron regulates their interaction will provide the first complete description of a translational control system in higher eukaryotic cells. In order to elucidate previously unknown components of the human cellular iron metabolism, we have established the mechanism for the uptake of iron in the genetically manipulatable simple eukaryote, Saccharomyces cerevisiae. This has led to the identification of a reductase/transporter system to explain the transmembrane, regulated uptake of iron in this organism.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 01600-06 CBMB |
|---|---|

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemical Basis of T Cell Activation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | L. E. Samelson | Med. Officer (Res.) | CBMB, NICHD |
| Others : | R. D. Klausner | Head | CBMB, NICHD |
| | M. L. Egerton | Visiting Fellow | CBMB, NICHD |
| | A-N Kong | IRTA | CBMB, NICHD |
| | J. N. Siegel | Senior Staff Fellow | CBMB, NICHD |
| | P. M. Thomas | Medical Staff Fellow | CBMB, NICHD |
| A.F. Phillips & | G. A. Sarosi | HHMI Scholar Programs | CBMB, NICHD |
| | Y. Minami | Adjunct Scientist | CBMB, NICHD |
| | K. W. Kim | Technician | CBMB, NICHD |

COOPERATING UNITS *(if any)*

Division of Cancer Biology and Diagnosis, NCI (A. Singer); Naval Medical Research Institute (C. June); USUHS(J.J. Mond).

LAB/BRANCH
Cell Biology and Metabolism Branch

SECTION
Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 7 | 6 | I |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Activation of the multicomponent antigen receptor in T cells (TCR) results in rapid activation of protein tyrosine and serine kinase pathways. The tyrosine kinase pathway has previously been shown to be activated before phospholipase C. We have used the tyrosine kinase inhibitor, herbimycin, to demonstrate that inhibition of tyrosine phosphorylation results in inhibition of TCR-mediated phosphoinositide hydrolysis. These results suggest that the tyrosine phosphorylation pathway regulates phospholipase C activity. Using digitonin as a detergent to solubilize a murine T cell hybridoma, we have demonstrated that the protein tyrosine kinase fyn is noncovalently associated with the TCR. This kinase may be responsible for the intracellular tyrosine phosphorylations detected after TCR engagement. In immature thymocytes, we detect a constitutive tyrosine phosphorylation of the TCR zeta chain. When the cells are removed from the thymus and cultured in suspension, the zeta chain dephosphorylates and TCR levels increase. These events correlate with an increased capability to respond to TCR ligation with increases in intracellular calcium. Ligation of surface immunoglobulin, the B cell antigen receptor, also results in an increase in tyrosine phosphorylation of intracellular substrates in B cells.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 01604-05 CBMB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interleukin-2 Receptors - Structure, Function, and Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | W.J. Leonard | Med. Officer (Res.) | CBMB, NICHD |
| Others: | S. Adelstein | Adjunct Technician | CBMB, NICHD |
| | M. Erdos | Biologist | CBMB, NICHD |
| | J. R. Gnarra | IRTA Fellow | CBMB, NICHD |
| | J. Lin | Adjunct Technician | CBMB, NICHD |
| | M. Napolitano | Visiting Fellow | CBMB, NICHD |
| | H. Otani | Visiting Fellow | CBMB, NICHD |
| | M. Toledano | Visiting Fellow | CBMB, NICHD |

COOPERATING UNITS *(if any)*

O. W. McBride, J. P. Siegel, W. Burgess, J. Pierce

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 7 | 6 | 1 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human interleukin-2 receptor is being studied to understand specific critical components of the T cell immune response in normal and neoplastic cells. We have significantly progressed in our study of (1) biochemical analysis of high, intermediate, and low affinity human and murine IL2Rs; (2) characterization of transcription regulatory sequences in the IL2 receptor alpha chain gene (3) the characterization of the transcription regulatory sequences in the IL-2 receptor beta chain gene; (4) the expression of the IL-2R alpha and beta chain cDNAs in various target cell types to assist in elucidating IL-2 mediated signal transduction. In addition, we have significantly progressed in our study of the Act-2 cytokine. We have (1) characterized the gene encoding the Act-2 cytokine and 2) the characterized cellular receptors for the Act-2 cytokine as a prelude to potentially cloning the Act-2 receptor.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 01601-06 CBMB |

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Aspects of the Regulation of the Human Transferrin Receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | J.B. Harford | Senior Investigator | CBMB, NICHD |
| Others: | J.L. Casey | IRTA Fellow | CBMB, NICHD |
| | D.M. Koeller | Medical Staff Fellow | CBMB, NICHD |
| | V.C. Ramin | Guest Worker | CBMB, NICHD |
| | J. A. Horowitz | IRTA Fellow | CBMB, NICHD |
| | R.D. Klausner | Head | CBMB, NICHD |

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Cell Biology and Metabolism Branch

SECTION

Section on Organelle and Receptor Structure and Function

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4 | 4 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Post-transcriptional regulation of transferrin receptor mRNA levels by iron is mediated by a portion of the 3' untranslated region (UTR) of the mRNA. We have previously shown that a 678 nucleotide fragment of the 3' UTR contains the regulatory element(s). Within this region are five RNA structures which resemble the iron responsive element (IRE) in the 5' untranslated region of the ferritin mRNA, which is regulated translationally by iron. The IRE's from the ferritin and transferrin receptor mRNA's compete in an in vitro assay for interaction with a cytoplasmic protein; the activity of this IRE-binding protein is dependent upon the iron status of the cells. Thus, despite the differences in the translational regulation of ferritin and the regulation of TfR mRNA levels, these two post-transcriptional regulatory mechanisms share a cis-acting RNA element (IRE) and a trans-acting cytoplasmic protein that interacts with the two mRNA's. We are attempting to understand more fully the mechanism of the regulation of TfR expression.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00151-15 ERRB |
|---|---|

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Gonadal and Placental Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, end institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | K. J. Catt | Head, SHR | ERRB, NICHD |
| Others: | C. Moretti | Adjunct Scientist | ERRB, NICHD |
| | J. Ohnishi | Guest Researcher | ERRB, NICHD |
| | A. Bagnato | Adjunct Scientist | ERRB, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.5 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous studies on the molecular basis of hormone action during granulosa cell differentiation in vitro have shown the importance of growth factors and peptide regulators, as well as gonadotropic hormones in the control of ovarian follicular maturation. Several hypothalamic peptides, including GnRH, vasopressin, and CRF have been found to act as local regulators of gonadal function. The 43 amino acid hypothalamic polypeptide, growth hormone releasing factor (GRF) is found in several peripheral sites as well as in the brain, and acts on a common VIP/GRF receptor in several tissues as well as on the specific GRF receptor of somatotrophs in the anterior pituitary gland. Treatment with GRF has been shown to stimulate follicular development when administered with FSH to infertile women who are resistant to conventional gonadotropin therapy. The synergistic action of GRF on ovarian function could reflect the known actions of growth hormone and IGF-I in the granulosa cell, but the presence of GRF in the gonads, and in rat and human ovarian follicular fluid, suggests that local actions of the hypothalamic peptide could also be involved in this process. In cultured granulosa cells, GRF and its potent analogs and derivatives were found to bind to high-affinity receptors that also recognized VIP. Both GRF and VIP stimulated cAMP production, and markedly potentiated FSH-induced cAMP production, as well as progesterone production, aromatase activity, and the expression of LH receptors. These findings indicate that GRF acts on a common VIP/GRF receptor to promote gonadotropin-induced maturation of ovarian granulosa cells, and suggest that the peptide's action in gonadotropin-resistant infertile women is at least partly mediated by this mechanism. In other studies, procedures for the measurement of renin and angiotensinogen gene expression in the granulosa cell were evaluated for use in studies on hormonal regulation of these components of the ovarian renin-angiotensin system in vitro. While solution hybridization procedures were applicable to the detection of mRNAs for renin and angiotensinogen, the low levels of these mRNAs necessitated the use of more sensitive methods for their quantitation in cultured granulosa cells. This is being performed by the polymerase chain reaction, using conditions optimized for amplification of granulosa-cell mRNA, employing an appropriate internal standard to permit quantitation of the amplified sequences and their changes during stimulation of granulosa cell maturation in vitro by gonadotropins and other regulatory factors.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00193-05 ERRB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Angiotensin II Receptors and Activation Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | K. J. Catt | Head, SHR | ERRB, NICHD |
| Others: | T. Balla | Visiting Scientist | ERRB, NICHD |
| | A. Clark | Visiting Scientist | ERRB, NICHD |
| | A. Baukal | Chemist, GS-12 | ERRB, NICHD |
| | K. Sandberg | IRTA Fellow | ERRB, NICHD |
| | C. Ambroz | NRSA Fellow | ERRB, NICHD |
| | H. Ji | Visiting Fellow | ERRB, NICHD |
| | J. Ely | Adjunct Scientist | ERRB, NICHD |

COOPERATING UNITS *(if any)*

Dept. of Physiology, Semmelweiss University Medical School, Budapest (L. Hunyady)
Contract for preparation of adrenal and pituitary cells ND1-HD-0-2806

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.5 | 6.5 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The properties and activation mechanisms of the angiotensin II (AII) receptor were studied in rat and bovine adrenal glomerulosa cells and in Xenopus laevis oocytes after expression of AII receptors from poly(A)+ mRNA isolated from adrenal glomerulosa cells and other AII target tissues. The inhibitory action of somatostatin on AII-stimulated aldosterone production in rat glomerulosa cells was found to be related to its Gi-mediated inhibition of basal cAMP levels in the glomerulosa cell rather than to any effect on cytoplasmic calcium levels. The stimulatory action of AII on phosphoinositide hydrolysis was associated not only with rapid production and degradation of Ins(1,4,5)P3, but also with short-term and marked long-term changes in several highly phosphorylated inositols. These included early increases in Ins(1,3,4,6)P4 and Ins (3,4,5,6)P4, as well as the Ins(1,3,4,5)P4 derived from Ins(14,5)P3, and progressive increases in Ins(1,3,4,6)P4 and Ins(3,4,5,6)P4, the precursors of InsP5. The cAMP-mediated secretagogue, ACTH, also enhanced AII-stimulated increases in the new InsP4 isomers, by increasing the activity of the 6-kinase enzyme. Thus, the reactions by which Ins(1,3,4,6)P4 and Ins(3,4,5,6)P4 are formed and converted to InsP5 are influenced by agonists acting through both calcium- and cAMP-dependent mechanisms. The conversion of InsP5 to InsP6 was demonstrated in Xenopus oocytes, providing the first evidence for this metabolic step in vertebrate cells. Analysis of the actions of Ins(1,4,5)P3 and Ins(1,3,4,5)P4 on calcium mobilization from adrenal microsomes revealed that the tetrakisphosphate stimulates calcium release from a discrete intracellular store and appears to operate through a receptor site that is distinct from the well characterized receptor for Ins(1,4,5)P3. The other intracellular messenger derived from AII-stimulated phosphoinositide hydrolysis, diacylglycerol (DAG), exhibited a biphasic increase in agonist-stimulated glomerulosa cells, concomitant with the formation of Ins(1,4,5)P3. The initial phases of both InsP3 and DAG responses to AII were not affected by the absence of extracellular calcium, whereas the second phase of the InsP3 response was markedly inhibited and DAG formation was only partially impaired. The retention of a substantial DAG response to AII in the absence of concomitant InsP3 formation indicates that a major proportion of the DAG produced during AII action is derived from sources other than phosphoinositides. Studies on the use of the Xenopus oocyte to express AII receptors from adrenal mRNA and to screen cDNA expression libraries revealed that AII stimulates calcium mobilization in follicular oocytes by acting on receptors present in the adherent follicle cells. The ability of AII to induce a calcium-mobilizing signal that is transferred to the oocyte through gap junctions, and accelerates its rate of maturation, suggests that AII may have a physiological role in the control of oocyte development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00193-05 ERRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Angiotensin II Receptors and Activation Mechanisms

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | K. J. Catt | Head, SHR | ERRB, NICHD |
| Others: | T. Balla | Visiting Scientist | ERRB, NICHD |
| | A. Clark | Visiting Scientist | ERRB, NICHD |
| | A. Baukal | Chemist, GS-12 | ERRB, NICHD |
| | K. Sandberg | IRTA Fellow | ERRB, NICHD |
| | C. Ambroz | NRSA Fellow | ERRB, NICHD |
| | H. Ji | Visiting Fellow | ERRB, NICHD |
| | J. Ely | Adjunct Scientist | ERRB, NICHD |

COOPERATING UNITS *(if any)*
Dept. of Physiology, Semmelweiss University Medical School, Budapest (L. Hunyady)
Contract for preparation of adrenal and pituitary cells ND1-HD-0-2806

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.5 | 6.5 | |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The properties and activation mechanisms of the angiotensin II (AII) receptor were studied in rat and bovine adrenal glomerulosa cells and in Xenopus laevis oocytes after expression of AII receptors from poly(A)+ mRNA isolated from adrenal glomerulosa cells and other AII target tissues. The inhibitory action of somatostatin on AII-stimulated aldosterone production in rat glomerulosa cells was found to be related to its Gi-mediated inhibition of basal cAMP levels in the glomerulosa cell rather than to any effect on cytoplasmic calcium levels. The stimulatory action of AII on phosphoinositide hydrolysis was associated not only with rapid production and degradation of Ins(1,4,5)P3, but also with short-term and marked long-term changes in several highly phosphorylated inositols. These included early increases in Ins(1,3,4,6)P4 and Ins (3,4,5,6)P4, as well as the Ins(1,3,4,5)P4 derived from Ins(14,5)P3, and progressive increases in Ins(1,3,4,6)P4 and Ins(3,4,5,6)P4, the precursors of InsP5. The cAMP-mediated secretagogue, ACTH, also enhanced AII-stimulated increases in the new InsP4 isomers, by increasing the activity of the G-kinase enzyme. Thus, the reactions by which Ins(1,3,4,6)P4 and Ins(3,4,5,6)P4 are formed and converted to InsP5 are influenced by agonists acting through both calcium- and cAMP-dependent mechanisms. The conversion of InsP5 to InsP6 was demonstrated in Xenopus oocytes, providing the first evidence for this metabolic step in vertebrate cells. Analysis of the actions of Ins(1,4,5)P3 and Ins(1,3,4,5)P4 on calcium mobilization from adrenal microsomes revealed that the tetrakisphosphate stimulates calcium release from a discrete intracellular store and appears to operate through a receptor site that is distinct from the well characterized receptor for Ins(1,4,5)P3. The other intracellular messenger derived from AII-stimulated phosphoinositide hydrolysis, diacylglycerol (DAG), exhibited a biphasic increase in agonist-stimulated glomerulosa cells, concomitant with the formation of Ins(1,4,5)P3. The initial phases of both InsP3 and DAG responses to AII were not affected by the absence of extracellular calcium, whereas the second phase of the InsP3 response was markedly inhibited and DAG formation was only partially impaired. The retention of a substantial DAG response to AII in the absence of concomitant InsP3 formation indicates that a major proportion of the DAG produced during AII action is derived from sources other than phosphoinositides. Studies on the use of the Xenopus oocyte to express AII receptors from adrenal mRNA and to screen cDNA expression libraries revealed that AII stimulates calcium mobilization in follicular oocytes by acting on receptors present in the adherent follicle cells. The ability of AII to induce a calcium-mobilizing signal that is transferred to the oocytes and accelerates its rate of maturation, suggests that AII may have a physiological role in the control of oocyte development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00184-12 ERRB

PERIOD COVERED

October 1, 1989 to September 29, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation of Pituitary Hormone Secretion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | K. J. Catt | Head SHR | ERRB, NICHD |
| Others: | S. Stojilkovic | Visiting Scientist | ERRB, NICHD |
| | T. Iida | Visiting Fellow | ERRB, NICHD |
| | F. Merelli | Visiting Fellow | ERRB, NICHD |
| | L. Zheng | Visiting Fellow | ERRB, NICHD |
| | L. Krsmanovic | Adjunct Scientist | ERRB, NICHD |
| | S. Al-Damluji | Adjunct Scientist | ERRB, NICHD |

COOPERATING UNITS *(if any)*

Lab of Cell Biology and Genetics, NIDDK (E. Rojas)
Contract for preparation of adrenal and pituitary cells NO1-HD-0-2806

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Hormonal Regulation

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6.0 | 6.0 | 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Gonadotropin releasing hormone (GnRH) is the primary hypothalamic regulator of gonadotropin biosynthesis and secretion in the anterior pituitary gland. Activation of the GnRH receptor causes rapid increases in phosphoinositide hydrolysis and calcium mobilization, and is followed by biphasic increases in cytoplasmic calcium ([Ca])$_i$ and LH secretion in gonadotroph-enriched pituitary cells. The initial phase of LH release depends largely on mobilization of intracellular calcium by $\text{Ins}(1,4,5)\text{P}_3$, and the sustained phase of hormone secretion requires continuous entry of extracellular calcium through plasma-membrane channels. The agonist-stimulated entry of calcium during the plateau phase of the cytosolic calcium response depends on calcium entry through both L-type and dihydropyridine-insensitive channels. Patch clamp analysis of the calcium currents in rat gonadotrophs revealed the presence of rapidly inactivating T-type channels and a delayed component with slower inactivation, corresponding to L-type channels. The latter are potentiated and inhibited by dihydropyridine agonist and antagonist analogs, and participate in agonist-mediated calcium influx and gonadotropin secretion. Depolarization-induced calcium entry through L-channels initially potentiates the agonist-induced release of calcium from intracellular stores, but attenuates the subsequent plateau phase of the response. The retention of an attenuated LH secretory response when the plateau phase of the cytoplasmic calcium response was abolished in the absence of extracellular calcium indicates that while influx and elevation of intracellular calcium are predominent factors in GnRH-induced hormone secretion, other elements of the calcium-phospholipid signaling system also contribute to the maintenance of LH release during agonist-receptor interaction. Protein kinase C, which was previously shown to participate in LH synthesis and secretion, was found to activate voltage- and dihydropyridine-sensitive calcium channels in cultured pituitary cells, but to exert inhibitory effects on cytoplasmic calcium levels during prolonged activation by phorbol esters. Thus, protein kinase C exerts both positive and negative control of the function of voltage-sensitive calcium channels during its participation in the mechanism of hormone secretion in pituitary gonadotrophs. The existence of additional regulators of gonadotropin secretion, acting via calcium-dependent mechanisms, was shown by findings on the abilities of GABA and endothelin to stimulate LH release from cultured gonadotrophs. Activation of GABA-A receptors promotes calcium influx through L channels, secondary to depolarization by increased chloride conductance. Endothelin also stimulated gonadotropin release, primarily by activating phosphoinositide hydrolysis with increased calcium mobilization and influx through L-type voltage sensitive calcium channels. These factors may serve as additional hypothalamic regulators of gonadotropin secretion from the pituitary gland.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00150-15 ERRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Gonadal Prolactin, LH/hCG Receptors and Gonadotropin Biological Activity.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.L. Dufau | Head, SME | ERRB, NICHD |
| Others: | C-H. Tsai-Morris | Sr. Staff Fellow | ERRB, NICHD |
| | Y. Funatsu | Visiting Associate | ERRB, NICHD |
| | E. Buczko | Adjunct Scientist | ERRB, NICHD |
| | C. Delgado | NRSA Fellow | ERRB, NICHD |
| | R. Zhang | Adjunct Scientist | ERRB, NICHD |
| | W. Wang | Adjunct Scientist | ERRB, NICHD |

COOPERATING UNITS *(if eny)*
Contract for preparation of gonadal cells and cell fractions HD-6-2904
University of Virginia, Dept. of Medicine, Charlottesville, VA (J. Veldhuis)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Molecular Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 3.9 | PROFESSIONAL: 3.4 | OTHER: 0.5 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Three prolactin receptor isoforms were isolated from the purified ovarian receptor, indicating structural heterogeneity of receptors possibly derived from the same gene. HPLC analysis and DEAE chromatography demonstrated three distinct ovarian lactogen receptor species with unique and highly reproducible retention times which migrated as single bands of 80, 40 and 34 kDa on SDS-PAGE. Subsequent studies demonstrated that these were not associated with thiol linkages. Immunological studies indicated homology among the three ovarian receptor forms and the rat liver receptor, and also demonstrated that the 80 kDa ovarian species contains an amino acid sequence that is not present in the 34 kDa and 40 kDa species or the rat liver receptor. The latter finding, coupled to the demonstration of a single 80 kDa protein species under reducing conditions, conclusively demonstrated that the 80 kDa form is not a dimer of the 34 kDa and 40 kDa species. This was also observed at the molecular level with the isolation and characterization of novel ovarian cDNA clones coding for distinct receptor isoforms. One of these is a membrane-anchored receptor bearing an extended and unique cytoplasmic domain with the potential for signal transduction and mitogenic activity. Another is a truncated receptor with a short cytoplasmic domain possibly involved in transport and/or clearance, and a third codes for a low Mr binding site capable for expression as a soluble protein.

Recent studies have demonstrated that the LH receptor gene contains several introns, located within the region coding for the extracellular domain of the molecule, which determine the nature and generation of LH receptor isoforms. By the isolation of a rat testicular cDNA clone (of deduced amino acid sequence lacking the transmembrane) followed by the expression of this cDNA as a soluble protein in COS1 cells it was demonstrated that the N-terminal extracellular regions of the LH receptor plays a major role in gonadotropin binding. These findings are of great interest because they demonstrate that the LH receptor is distinct from most other G-protein-coupled receptors, which are intronless and contain their binding domains within the transmembrane region rather than the extracellular domain. In other studies correlation between changes of LH receptor number and steady state levels of LH receptor mRNAs (5.8, 2.6 and 2.3 kb) have demonstrated that the expression of LH receptors induced during follicular maturation, ovulation and desensitization is related to prevailing levels of LH receptor mRNA in the ovary.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00149-14 ERRB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bioassay of Serum Luteinizing Hormone (LH) and Chorionic Gonadotropin

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.L. Dufau | Head, SME | ERRB, NICHD |

COOPERATING UNITS *(if any)*

University of Virginia, Dept. of Medicine, Charlottesville, VA (J. Veldhuis)
Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

COMBINED WITH Z01 HD 00150-15 ERRB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00147-15 ERRB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Action of Peptide Hormones in Steroidogenic Cells.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | M.L. Dufau | Head, SME | ERRB, NICHD |
| Others: | M. Kitamura | Visiting Fellow | ERRB, NICHD |
| | M. Hirabayashi | Visiting Fellow | ERRB, NICHD |
| | S. Ulisse | Adjunct Scientist | ERRB, NICHD |
| | A. Fabbri | Adjunct Scientist | ERRB, NICHD |
| | A. Khanum | Adjunct Scientist | ERRB, NICHD |
| | J. Tinajero | Visiting Associate | ERRB, NICHD |
| | Y. Funatsu | Visiting Associate | ERRB, NICHD |

COOPERATING UNITS *(if any)*

Contract for preparation of gonadal cells and cell fractions HD-6-2904

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Molecular Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.0 | 2.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Multiple hormonal controls are required to modulate the primary actions of gonadotropin in the Leydig cell (i.e. estrogen, intracellularly; CRF and angiotensin II, at the membrane level). The presence of a unique CRF receptor and its mechanisms of action in the Leydig cell has been elucidated. CRF acts rapidly (minutes) in the Leydig cell to exert highly effective negative autocrine regulation on Leydig cells. The inhibitory actions of CRF on cAMP generation and steroidogenesis are mediated by interaction of the peptide with a distinct high affinity receptor subtype. Although, present in low abundance in the Leydig cells (500 to 800/cell for CRF vs 20,000 for LH/hCG and 2,000/cell for AII) CRF receptors are highly coupled to a G-unit other than Gs and Gi (possibly Gp). CRF caused marked inhibition of $Mn^{2+}$ and forskolin stimulated adenylate cyclase activity in detergent solubilized membranes indicating that the site of inhibition indicated by the peptide is the catalytic subunit of adenylate cyclase. The inhibitory action of CRF is prevented by inhibitors of PKC and the action of the peptide was not observed in cells depleted of PKC. Furthermore, CRF caused rapid translocation of PKC. Thus, these studies demonstrate that the inhibitory action of CRF is exerted through direct or indirect actions of PKC on the catalytic unit of adenylate cyclase. Regulation of CRF release from the Leydig cell is modulated directly or indirectly through the gonadotropin stimulus, which releases the peptide in a dose-dependent fashion. The action of the trophic hormone is reproduced by cyclic AMP analogs. In contrast, norepinephine, while effective in stimulating cAMP and testosterone production, did not cause CRF release and forskolin was less effective than the trophic stimulus. This indicates that gonadotropin stimulation of CRF release is highly compartmentalized. Studies using the $\alpha$-helical CRF (9-41) antagonist revealed tonic supression of basal and gonadotropin-stimulated testosterone production by endogenously produced CRF. The CRF antagonist, and also an antibody to CRF, increased cell sensitivity to the trophic hormone. The intracellular pool of CRF in the cells is large and the minor proportion of peptide released during stimulation acts as a potent autocrine regulator of gonadotropin action. These findings have led to the proposal of CRF as a local antireproductive hormone responsible for certain disorders of androgen production and some forms of infertility in men. In other studies, we have provided evidence of yet another effect of forskolin in Leydig cells by the demonstration of an inhibitory action of high doses of this diterpene on hCG-stimulated steroidogenesis while potentiating hCG-stimulated adenylate cyclase activity and cAMP production. Further, this inhibitory effect was cAMP-independent and located beyond pregnenolone synthesis. The site of inhibition of forskolin seems to reside at the conversion of androstenedione to testosterone, possibly with reduced affinity of 17$\alpha$-hydroxysteroid dehydrogenase.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00035-18 ERRB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)

The Structure and Function of Biologically Active Molecules

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator.*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| PI: | H.C. Chen | Head, SMSPC | ERRB, NICHD |
| Others: | J.L. Morell | Research Chemist | ERRB, NICHD |
| | J.H. Brown | Research Chemist | ERRB, NICHD |

COOPERATING UNITS (*if any*)

Department of Pathology, Thomas Jefferson University Hospital (C.M. Huang)
Laboratory of Immunology, NIDR (W.A. Hook)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 2.5 | PROFESSIONAL: 2.5 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

Studies on structural design, chemical synthesis, conformational properties and the mode of action of vertebrate peptide antibiotics, magainin are the focus of this program. We prepared all D amino acid analogues of our most active magainin peptides by the solid-phase method. The peptides designated as Endin A, B, F and G gave identical elution characteristics in reverse phase HPLC and mirror images in the CD spectra to their counterparts. Endin F as a representative was completely resistant to pronase digestion under conditions in which magainin F was totally degraded. The Endins were only 2-fold more active antibacterially. No difference in the spectrum of biological activity was observed. These results suggest that the proteolytic enzyme susceptibility has limited effect toward the increase of antibacterial activity. As it is expected, all D amino acid substituted analogues will exhibit in left handed helical form the same relationship between hydrophobic and hydrophilic faces as is observed in the L peptides. Thus, it appears that the helical amphiphilic conformation to provide correct interaction with lipid membrane is important in determining biological activities. Moreover, the mode of action of these peptides is unlikely to involve a stereospecific entity such as receptor or enzyme directly. The in vitro activities of magainin B, G and H against pathogenic protozoa Blastocystis hominis, Entamoeba histolytica and Trypanosoma cruzi were assessed for their morphological integrity and motility. The antiprotozoan activities were B>G>H the same order as the antibacterial activity. The parallelism between these two activities observed in these three analogues suggest that the helix structure of magainins is important in eliciting lysis activity on protozoa. Studies were also performed with seven highly potent all L-form analogues and seven D-Ala permuted analogues for their histamine-release (HR) activity in rat peritoneal mast cells. Magainins and analogues yielded low to moderate activity. In three duplicate experiments, the results have indicated consistently an inverse relationship between antimicrobial and the HR activity. Therefore, it may suggest that an α-helical conformation is not only unimportant but also unfavorable and the formation of anion channels is not necessary to achieve HR from mast cells. These studies indicate that magainin analogues not only have a practical therapeutic value in the treatment of infection and diseases in man and animals but also provide a model to correlate structure and thermodynamic variables with biological activities, and to understand unique properties related to peptide-phospholipid interaction.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00146-15 ERRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Structural Studies of Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | H.C. Chen | Head, SMSPC | ERRB, NICHD |
| Others: | J.H. Brown | Research Chemist | ERRB, NICHD |

COOPERATING UNITS *(if any)*

Department of Biochemistry, New York University School of Medicine (S. Lee-Huang)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Molecular Structure and Protein Chemistry

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 0.5 | PROFESSIONAL: 0.5 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project focuses on the study of physico-chemical characteristics, and structural properties of proteins in order to elucidate different molecular forms and structural requirements for various biological activities. During this reporting period, a medicinal plant protein from the seeds and fruits of Momordica charantia, designated as MAP 30 or Momordica anti-human immunodeficiency virus (HIV) protein, was studied. This protein has been purified to homogeneity, is basic, 30 kDa in size, and exhibits dose-dependent inhibition of cell-free HIV-1 infection and replication. The sequence analysis of the amino-terminal 44 amino acid residues of MAP 30, and of the closely related and functionally similar medicinal plant proteins, trichosanthins, have revealed 34% and 57% sequence homology to ricin A chain and trichosanthin, respectively, when both identical and conserved residues are taken into account for comparison. Consistent with this finding, MAP also inhibits eukaryotic translation in vitro. However, unlike ricin and trichosanthin, this new protein is not toxic to intact host cells at the dose levels at which HIV infection and replication experiments were performed. Tricosanthins are now in the phase 2 clinical evaluation for the treatment of acquired immunodeficiency syndrome (AIDS). The available data suggest that MAP 30 may be a superior therapeutic agent in the treatment of HIV infections. For the future course of the project, complete primary protein structure will be elucidated and attempts will be made to understand secondary and tertiary structures that will be essential in the design of analogues and the understanding of structure-function relationships. Furthermore, target-oriented MAP-CD4 or MAP-antiCD4 antibody complexes will be prepared in order to increase the efficacy of the protein in the treatment of AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01HD00187-11ERRB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Hormonal Regulation of Cellular Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | K.-P. Huang | Head, SMR | ERRB, NICHD |
| Others: | F. Huang | Expert | ERRB, NICHD |
| | H. Nakabayashi | Visiting Associate | ERRB, NICHD |
| | K.H. Chen | IRTA Fellow | ERRB, NICHD |
| | C.W. Mahoney | NRC Fellow | ERRB, NICHD |
| | F.M.J. Heemskerk | Visiting Fellow | ERRB, NICHD |

COOPERATING UNITS *(if any)*
Lab of Chemical Pharmacology, NHLBI, NIH (M.A. Beaven); Lab of Cell Biology, MH, NIH (W.S. Young); Lab of Biochemistry, NCI, NIH (S.H. Wilson); Biological Psychiatry Branch, MH, NIH (D.-M. Chuang); Carnegie Institute of Washington, Baltimore, MD (S.L. McKnight); Centre National De La Recherche Scientifique, Strasbourg, France (A.N. Malviya)

LAB/BRANCH
Endocrinology and Reproduction Research Branch

SECTION
Section on Metabolic Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 6 | 6 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Phosphorylation-dephosphorylation of proteins is one of the most important mechanisms for the regulation of cellular functions. Protein kinase C (PKC), a Ca2+/phospholipid-dependent protein kinase, has emerged as a pivotal regulatory element for cell growth, differentiation, gene expression, hormone secretion, cell surface receptor function, and cellular metabolism. This protein kinase can be activated by diacylglycerol, a second messenger generated by signal-induced breakdown of phosphoinositides. In addition, it has been identified as a receptor for tumor-promoting phorbol esters which elicit pleiotropic responses comparable to those stimulated by many hormones and growth factors. Three isozymic forms of PKC have been identified from mammalian brains. Polyclonal and monoclonal antibodies against these enzymes were prepared for their immunochemical characterization. These enzymes were found to have distinct tissue, cellular, and subcellular distributions and were differentially expressed during development. The role of PKC in mediating the action of neurotransmitter was investigated with neurons grown in culture. These cells express PKCII and III in a similar growth-regulated pattern as in intact cerebella of postnatal rats. Stimulation of the cultured granule cells with the excitatory amino acid, glutamate, or phorbol ester causes the association of both PKCs with the particulate fraction; however, only PKCII, but not PKCIII, was found to associate with the membrane skeleton. Membrane association of PKC in the cell is an obligatory step for activation. Several inositol-containing phospholipids were found to interact with PKC with high affinity, in particular, the CNS-specific PKCI. Interaction of PKC with these phospholipids places the enzyme in close proximity to diacylglycerol generated by the activation of phospholipase C. This class of phospholipids may serve as anchoring sites of PKC at the membrane. Activation of PKC in the control of gene expression was investigated by studying the phosphorylation of a transcription factor, CCAAT/enhancer-binding protein (CEBP), which contains a leucine-repeat dimerization interface and an upstream basic region for DNA binding. Phosphorylation of a truncated form of CEBP at two Ser residues within the basic region results in attenuation of DNA binding. Mutation of one of the Ser residues to Cys obviates the PKC-mediated response, suggesting a role of PKC in transcriptional control. The genomic structure of PKC I gene was analyzed for the purpose of defining tissue-specific and development-regulated expression of this kinase. A genomic fragment containing the 5'-flanking region, the first exon, and the first intron, has been isolated and sequenced. Several potential transcriptional factor binding sites have been identified by footprint analysis.

GPO 914-918

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01HD00194-02ERRB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

ACTH Regulation of Adrenocortical Function (Steroidogenesis): Biochemistry & Molecular Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | C.A. Strott | Head, SACB | ERRB, NICHD |
| Others: | Y.C. Lee | Chemist | ERRB, NICHD |
| | W.J. Driscoll | IRTA | ERRB, NICHD |
| | T. Oeda | Visiting Fellow | ERRB, NICHD |

COOPERATING UNITS *(if any)*

Laboratory of Neurochemistry, NINDS (Dr. Mark Whitnall)

LAB/BRANCH

Endocrinology and Reproduction Research Branch

SECTION

Section on Adrenal Cell Biology (SACB)

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, MD 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 4.0 | PROFESSIONAL: 4.0 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The steroidogenic response to ACTH can be separated into acute and subacute aspects. The acute response (sec-min) occurs at the level of the mitochondrion and involves the translocation of cholesterol to the inner mitochondrial membrane followed by the conversion of cholesterol to pregnenolone (rate-limiting). The subacute response (hrs) occurs at the level of the genome and leads to the synthesis of steroidogenic enzymes and cofactors. Both the acute and subacute responses to ACTH are mediated, inpart, by cAMP, and both are dependent on the *de novo* synthesis of protein. A steroidogenic regulatory protein has been proposed but none has been identified as yet. The role of Ca$^{2+}$ in ACTH action is complex; Ca$^{2+}$-regulated protein kinases and/or phosphatases may be involved. Pregnenolone, the product of the rate-limiting step in steroidogenesis, is poorly soluble in aqueous media and must be translocated between intracellular compartments. Progesterone, which is derived from pregnenolone and closely related, not only serves as a key steroid intermediate, but is also a ligand for a member of the steroid receptor super-family.

To examine the complex process of ACTH action, specific intra-adrenal steroid-binding proteins are being investigated. Two such proteins have been detected and characterized by the SACB laboratory: 1) a cytoplasmic pregnenolone-binding protein ($P_5$BP), and 2) a nuclear progesterone-binding protein ($P_4$BP). The $P_5$BP has a $M_r$ of 34 kDa and exists as charge isoforms with PI's of 6.4, 5.5, 5.4 and 5.2. The PI 5.4 isoform may represent the active binding molecule. The binding of pregnenolone to the heat-labile $P_5$BP is regulated by a phosphorylation/dephosphorylation system, and requires a heat-stable cofactor. The $P_5$BP kinase has been partially purified and has been found to be absolutely dependent on Ca$^{2+}$. $P_5$BP is present only in cells regulated by ACTH and not in steroidogenic cells regulated by other peptide hormones. The content of $P_5$BP in fasciculata cells is increased by ACTH. These findings suggest a functional relationship between $P_5$BP and ACTH. The $P_5$BP has been purified and internal peptide sequences determined following partial tryptic digestion. A full-length $P_5$BP cDNA is currently being cloned.

The $P_4$BP has been purified 2000-fold by steroid-affinity chromatography, and photoaffinity labeling has revealed a $M_r$ of 50 kDa. Biochemical and immunological evidence indicate that $P_4$BP is distinct from the classical progesterone receptor which has a Mr of 75 to 115 kDa depending on the species examined. The $P_4$BP has been found to bind *in vitro* to DNA suggesting a transcriptional regulatory role. $P_4$BP synthesis is stimulated *in vivo* by ACTH further suggesting a mediating role in ACTH action.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD-00071-18-LMGD

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene and Transgene Regulation in the Developing Mouse

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

H. Westphal, Chief, LMGD
E.M. Füchtbauer, Visiting Fellow/Guest Researcher
S.-P. Huang, Chemist
E. Lee, Veterinarian
P. Love, Medical Staff Fellow
A. Miller, Biologist
B. Mosinger, Visiting Fellow
U. Tillmann,Guest Researcher/
Visiting Fellow
A. Titomirov, Visiting
Associate
M. Tremblay, Guest Researher/
Visiting Associate
Y. Yaginuma, Visiting Fellow

COOPERATING UNITS *(if any)*

LMB/NIDDK - G. Felsenfeld
NSB/NIMH - E. Ginns
LHB/NHLBI - A. Nienhuis
LDMI/NICHD - K. Ozato
LMDB/NEI - J. Piatigorsky
MCNE/NIDDK - B. Weintraub

LAB/BRANCH
Laboratory of Mammalian Genes and Development

SECTION
Section on Transgene Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 11.0 | 9 | 2 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular genetics of mammalian differentiation and oncogenesis studied in transgenic mice. The project comprises several approaches, some of them reflecting collaborations with other laboratories.

1. Oncogenesis of the embryonic lens. The embryonic lens vesicle undergoes a well defined fiber differentiation program, starting at mid gestation, that ultimately results in the formation of the translucent adult lens structure. We have targeted the expression of the large tumor antigens of the DNA viruses polyoma and SV40 to the developing lens in an effort to understand how these oncogene products interfere with the orderly process of lens differentiation. The polyoma T antigen immortalizes but does not transform lens cells. Its action is partly abrogated by basic fibroblast growth factor and by insulin, factors that have been implicated in normal lens differentiation.. By contrast, the SV40 T antigen causes lens tumors. The rate of tumor progression differs between individual lines of transgenic mice and appears to be linked to the state of differentiation attained in the embryonic lens at the time of T antigen accumulation. The results obtained with the two viral tumor antigens thus underscore the importance of the differentiating cell as the site where oncogene products compete with regulators of cell growth for the control of the cell cycle and where the fate of the organism is at stake. The availability of immortalized lens cells has enabled us to clone and characterize a gene that specifies a factor implicated in the regulation of lens-specific crystallin expression.

2. Gene targeting by homologous recombination. The purpose of this aspect of the project is to alter resident chromosomal genes of the mouse and to characterize the resulting mutant phenotypes. Following a technically demanding sequence of experimental steps, a wild type gene sequence is replaced in a pluripotant embryonic stem (ES) cell by inserting a mutated sequence in its place. The mutant ES cell is injected into a blastocyst which is transferred into a foster mother in an effort to obtain germ line chimeras that can propagate the mutant trait. We have established this technique in the laboratory and are presently targeting the c-fos proto-oncogene. In addition, we have joined collaborative efforts aimed at generating a mouse model of Gaucher's disease, a human disorder based on defined mutations of the glucocerebrosidase gene.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01-HD-01801-01-LMGD |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Molecular Analysis of Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
K.A. Mahon, Ph.D., Senior Staff Fellow
L.S. Amesse, M.D., Ph.D., Medical Staff Fellow
E. Hermesz, Visiting Associate
M.D. Johnson, M.D., Medical Staff Fellow
G.W. Robinson, Ph.D., NRC Senior Associate

COOPERATING UNITS *(if any)*
Center for Biologics Evaluation and Research, FDA (M.Jamrich)
Laboratory of Pathology, NCI (S. Mackem)

LAB/BRANCH
Laboratory of Mammalian Genes and Development

SECTION
Unit on Developmental Gene Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS:<br>3.4 | PROFESSIONAL:<br>2.9 | OTHER:<br>0.5 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are utilizing two approaches to identify and clone genes involved in the control of early development in the mouse.

Homeobox genes. Embryonic patterning in Drosophila is controlled by a regulatory gene network. Many of the genes included in this network contain conserved sequence motifs such as the homeobox. The expression pattern of many of the murine homeobox (Hox) genes suggests that a similar gene network may control development in this organism as well. However, the majority of the Hox genes that have been described are expressed relatively late during development (after gastrulation), and are probably not involved with very early determinative decisions. To investigate the role of homeobox genes in these early events, we have screened early embryonic cDNA libraries for homeobox genes that are specifically expressed during the very early stages of mouse embryogenesis (fertilization through early postimplantation development) using the polymerase chain reaction (PCR) technique and degenerate homeobox-specific oligonucleotides. We have cloned at least three new homeobox containing genes, and have shown by in situ hybridization that these genes are differentially expressed in the embryo during early postimplantation development. We have also identified a new homeobox gene family in the mouse with homology to the Distal-less gene of Drosophila, a gene necessary for development of the limbs of the fly. After determining the developmental expression profile for each gene, we plan to conduct expression studies in embryonic stem (ES) cells and transgenic mice to investigate their developmental functions.

Insertional mutagenesis via enhancer trapping. To identify new genes involved in development we have introduced enhancerless beta-galactosidase (lacZ) constructs into ES cells. Expression is only detectable if the construct has integrated into an active gene region. We have screened for cells that show interesting patterns of lacZ expression in differentiating embryoid bodies and in chimeras, and have obtained several lines that show either activation or inactivation of expression as differentiation occurs. Breeding of chimeric mice obtained from these cells will potentially lead to the derivation of mouse mutants at these loci. The interrupted genes can easily be cloned by virtue of the molecularly distinguishable lacZ sequences.

The significance of this work lies in the potential to study the largely inaccessible period of early mammalian embryogenesis on the molecular level. This will ultimately allow a better understanding of both normal and abnormal development.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01-HD-01800-02 LMGD |

PERIOD COVERED
January 1, 1990 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Analysis of Murine Pax Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
G.R. Dressler, Senior Staff Fellow, LMGD, NICHD
V. Steele-Perkins, Biologist, LMGD, NICHD

COOPERATING UNITS *(if any)*
LMGD (H. Westphal, E. Lee)
University of Otago, New Zealand (A. Reeve, M. Eccles)

LAB/BRANCH
Laboratory of Mammalian Genes and Development

SECTION
Unit on Molecular Embryology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER. |
|---|---|---|
| 1.0 | 0.75 | 0.25 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The induction and development of the mouse kidney is used to model cellular differentiation and morphogenesis. A newly identified gene, Pax2, is a prime candidate for controling early developmental processes, because of its homology to known developmental control factors of the fruitfly and its embryo specific expression pattern. How the Pax2 protein functions during early kidney development and its potential role in renal tumors is of prime interest. Two major avenues are currently being persued:

A. Characterization and functional analysis of the Pax2 gene product. Pax2 is transiently expressed in the metanephric blastema shortly after early kidney induction. In addition, Pax2 is also expressed in Wilms tumor, a common nephroblastoma. These data suggest a function for Pax2 in early kidney epithelium formation or in cellular polarization. Pax2 is a nuclear protein with sequence homology to certain types of transcription factors.

The potential of Pax2 to activate transcription by DNA binding or by protein-protein interactions are being investigated using a variety of biochemical methods.

B. Identification of induction responsive regulatory elements. The Pax2 promoter activates transcription in response to an intercellular contact event between mesenchyme and epithelium. The DNA sequences that mediate transcription by binding a specific induction activated factor are being delineated. It is hypothesized that factors modified post-tranlationally will transcriptionally activate Pax2 upon induction. Identification of these types of factors will help elucidate the signal transduction mechanism of an important morphogenetic event and may model similar inductive interactions throughout development.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00131-16 HGB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Human Biochemical Genetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | William A. Gahl | Head | HGB, NICHD |
| Others: | Isa M. Bernardini | Chemist | HGB, NICHD |
| | Megan D. Adamson | MSF Interinstitute | HGB, NICHD |
| | Hans C. Andersson | Medical Staff Fellow | HGB, NICHD |
| | Raili Seppala | Visiting Associate | HGB, NICHD |
| | Henk J. Blom | Visiting Fellow | HGB, NICHD |

Continued on Page 2

COOPERATING UNITS *(if any)*

See Attached

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Human Biochemical Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 8.0 | 7.0 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Seventy-five cystinosis patients are seen in the Human Genetics Branch and most are treated with cysteamine or phosphocysteamine. In pre-transplant patients, cysteamine therapy is maintaining renal function and assisting growth. In post-transplant patients, complications of long-term cystine accumulation are described. These include myopathy, neurological involvement, and severe swallowing difficulties. Cysteamine eyedrops (0.5%) dissolve corneal cystine crystals in young children and remove the haziness from the eyes of older children, with relief of photophobia.
2. Impaired lysosomal egress of free sialic acid has been demonstrated as the basic defect in Infantile Free Sialic Acid Storage Disease (ISSD). ISSD fibroblasts also store glucuronic acid due to impaired egress out of the lysosome.
3. The basic defect in sialuria has been demonstrated to be impaired feedback inhibition by CMP sialic acid of UDP N-acetylglucosamine 2-epimerase. Cytosolic free sialic acid levels can be reduced by treatment of the fibroblasts with cytidine.
4. The human kidney filters but does not reabsorb free sialic acid. This was demonstrated by studying patients with different filtered loads of free sialic acid.
5. Copper metabolism is impaired in Menkes' disease and Indian Childhood Cirrhosis cells. A 23 Kd protein with copper-binding activity is being characterized using normal human fibroblasts. Copper histidinate therapy of Menkes' disease is being pursued by a clinical protocol.
6. Unknown lysosomal storage disorders are investigated by carbohydrate analysis performed by pulsed ampermetric detection, and by lipid analysis performed by HPLC separation and Varex analysis.
7. Lysosomal membrane carriers are being investigated by reconstitution of proteoliposomes to be used as a test system for functional transport.

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|---|---|---|---|
| Others: | Thomas C. Markello | Medical Staff Fellow | HGB, NICHD |
| | Stephen G. Kaler | Medical Staff Fellow | HGB, NICHD |
| | Donna M. Krasnewich | MSF Interinstitute | HGB, NICHD |
| | Si Houn Hahn | NRSA Fellow | HGB, NICHD |
| | Satoru Oshiro | Visiting Scientist | HGB, NICHD |

Cooperating Units:

F. Tietze, Laboratory of Molecular and Cellular Biology, NIDDK
S. Mudd, Laboratory of General and Comparative Biochemistry, NIMH
J. Schneider, University of California at San Diego
J. Thoene, University of Michigan
G. Thomas, Johns Hopkins University
W. Rizzo, Medical College of Virginia
M. Kaiser-Kupfer, Clinical Branch, NEI
J. Schulman, IVF Institute, Fairfax, Virginia
M. Dalakas, Laboratory of Medical Neurology, NINCDS
J. Finkelstein, VA Hospital, Washington, D.C.
A. Tangerman, Nijmegen, The Netherlands
J. Fink, Laboratory of Developmental and Metabolic Neurology, NINCDS
L. Kohn, Laboratory of Biochemistry and Metabolism, NIDDK
G. Reed, Laboratory of Epidemiology and Biometry, NIAID
M. Datiles, Clinical Branch, NEI
Z. Goodman, Laboratory of Hepatic Pathology, AFIP
J. Olson, Johns Hopkins Hospital
L. Plotnick, Johns Hopkins Hospital
R. Reiss, Ohio State University at Columbus
P. Ozand, King Faisal Specialist Hospital and Research Centre, Saudi Arabia
L. Charnas, Laboratory of Human Genetics, NICHD
J. Hopwood, Adelaide Children's Hospital, Australia
C. Oliver, Laboratory of Microbiology and Immunology, NIDR
V. Chaudhry, Clinical Center, NIH
B. Sonies, Clinical Center, NIH
L. Racusan, Johns Hopkins Hospital
G. Ashwell, Laboratory of Biochemistry and Metabolism, NIDDK
G. Barsh, University of California-San Francisco
M. Renlund, Helsinki Children's Hospital, Finland
M. Evans, Wayne State University, Detroit, MI
K. Nicolaides, King's College School of Medicine, London
N. Papadopoulos, Clinical Center, NIH
D. Goldstein, Laboratory of Hypertension-Endocrine, NHLBI
D. Hamer, Laboratory of Cancer Biology and Diagnosis, NCI
M. Johnson, Laboratory of Molecular Genetics, NICHD
D. Rader, Laboratory of Molecular Disease, NHLBI
J. Hoeg, Laboratory of Molecular Disease, NHLBI
V. Reznik, University of California - San Diego
J. Handler, Johns Hopkins University

| | |
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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00133-13 HGB |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Glycogen Storage Disease and the Effect of Dichloracetate in Type Ia Glycogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: James B. Sidbury Adjunct Scientist HGB, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Theoretical and Physical Biology, NICHD (N. Esteban and A.L. Yergey).
Nutrition Department, CC (P. Brye)

LAB/BRANCH
Human Genetics Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.5 | 1.5 | 0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The glycemic response to the administration of modified corn starch and amylopectin compared with the unmodified form has consistently shown a lesser glycemic response to the modified forms of glycogen.

The accumulated evidence convincingly points to a beneficial effect on kidney function when the blood glucose level is maintained with corn starch. If long-term observations support this impression, corn starch therapy will have a significant long-term impact on prognosis of type I glycogen storage disease (GSD).

This study is designed to determine the rate of glucose production by the liver in individuals with type I glycogenosis. Patients with type III GSD were studied as control GSD subjects. The calculations of the latest data is not yet complete.

The pharmacokinetics and the effect of dichloracetate in patients with type Ia glycogenosis were studied in 3 patients.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00137-16 HGB |
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PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Regulation and Expression of the UDP-Glucuronosyltransferase Gene Family

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Ida S. Owens | Head | HGB, NICHD |
| Others: | Joseph Ritter | Senior Staff Fellow | HGB, NICHD |
| | Fan Chen | Visiting Fellow | HGB, NICHD |
| | Yhun Yhong Sheen | Adjunct Scientist | HGB, NICHD |
| | Huu Tran | Co-op Student | HGB, NICHD |
| | Phuong Nguyen | Biological Aid | HGB, NICHD |
| | Serena Farquharson | Biological Aid | HGB, NICHD |

COOPERATING UNITS *(if any)*

Brigham and Women's Hospital, Boston, MA (James Crawford), Frederick Cancer Research Facility, Frederick, MD (Ronald Lubet).

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Genetic Disorders of Drug Metabolism

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.4 | 3.4 | 1.0 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The isolation of new and critical human UDP-Glucuronosyltransferases has been pursued by using transferase-specific cDNAs as well as by using a 50-nucleotide conserved sequence. We have isolated two human liver cDNAs, HUG-Br1 and HUG-Br2, which encode proteins of 533 and 534 amino acids respectively, responsible for glucuronidating the 1XalphaC8 and 1XalphaC12 isomers of bilirubin to the respective mono- and to the diconjugate. The two encoded proteins have an identical carboxy terminus after amino acid residue 287, but are only 66% similar in the amino terminus. The overall similarity is 82%. The HUG-Br1 encoded message (2.6 kb) is high abundant, and that of HUG-Br2 (also 2.6 kb) is low abundant in human liver. The HUG-Br2 message is regulated by phenobarbital in at least one Old World monkey. Both messages, at 2.6 kb-length, are present in the explanted liver of a Type I Crigler-Najjar patient, but the level of that for HUG-BR1 is reduced several fold. The human liver transferase cDNA, UDPGT h-2, encoding the activity responsible for the conjugation of the critical bile acid, hyodeoxycholic acid, in humans has been identified. Previously, this isoform was shown to conjugate the 3, 4 catechol estrogens and estriols. We show also that a highly homologous form encoded by UDPGT h-1 has 1% the activity for the same three substrates. Both versions are induced in monkey by phenobarbital treatment. The significance of these results is that we can now isolate and characterize the normal human bilirubin transferase gene(s) and also that from the genome of the Type I Crigler-Najjar patient in order to determine its defect(s). The results should provide a DNA agent and critical information concerning possible gene therapy in the appropriate animal model or therapy otherwise for defective bilirubin transferase involved in several human syndromes.

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| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00404-08 HGB |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jean DeBrohun Butler | Senior Investigator | HGB, NICHD |
| Others: | Monique Powell | Biology Aid | HGB, NICHD |
| | Priya Sudarsanam | Summer IRTA Fellow | HGB, NICHD |

COOPERATING UNITS *(if any)*
Laboratory of Developmental and Metabolic Neurology, NINCDS (P. Pentchev); Environmental Protection Agency (S. Padilla); Laboratory of Cell Biology and Genetics, NIDDK (Mark Levine); Laboratory of Molecular and Cellular Biology, NIDDK (F. Tietze).

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Human Biochemical Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.25 | 1.0 | 0.25 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Continued studies of mutant mouse which stores cystine in lysosomes as do cystinotic patients; anamolies in cholesterol metabolism uncovered in Niemann-Pick C cells which show lysosomal storage of cholesterol and lack of intracellular cholesterol esterification. Investigation of effects of various cystine lowering drugs on levels of stored cystine in the mouse organs since there has not previously been an animal model for cystinosis.

2. Continued studies of cholesterol metabolism and transport in Niemann-Pick C and cystinotic fibroblasts. Investigation of the movement of cholesterol from the lysosomal compartment (where it is stored in Niemann-Pick C disease and in the mutant mouse) to the microsomes where cholesterol can be esterified and packaged for storage in lipid vesicles.

3. Characterization of cystinotic cell protein associated with cystine and present in a 2-fold excess in cystinotic versus normal fibroblasts. Amino acid analysis of this protein, N-terminal sequencing and a search for similar proteins. Since this protein has no covalently bound cystine, investigation of its cystine binding characteristics will be initiated. Findings will be compared with the known binding properties of the cystine binding protein from E. coli used in our laboratory to measure cystine levels in cystinotic cells.

4. Investigation of alternative ways of depleting cystine levels in cystinotic fibroblasts, using single and combined drugs that influence cystine levels in cystinotic fibroblasts.

5. Investigation of metabolism of ascorbic acid in normal and cystinotic fibroblasts. Continuation of measuring the kinetics of uptake of ascorbic acid by normal and cystinotic fibroblasts, followed by study of the effects this uptake has on the production of type I collagen, a major product of skin fibroblasts.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00404-08 HGB |
|---|---|

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell and Sulfur Metabolism in Fibroblasts of Genetic Diseases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Jean DeBrohun Butler | Senior Investigator | HGB, NICHD |
| Others: | Monique Powell | Biology Aid | HGB, NICHD |
| | Priya Sudarsanam | Summer IRTA Fellow | HGB, NICHD |

COOPERATING UNITS *(if any)*

Laboratory of Developmental and Metabolic Neurology, NINCDS (P. Pentchev); Environmental Protection Agency (S. Padilla); Laboratory of Cell Biology and Genetics, NIDDK (Mark Levine); Laboratory of Molecular and Cellular Biology, NIDDK (F. Tietze).

LAB/BRANCH

Human Genetics Branch

SECTION

Section on Human Biochemical Genetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.25 | 1.0 | 0.25 |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

1. Continued studies of mutant mouse which stores cystine in lysosomes as do cystinotic patients; anamolies in cholesterol metabolism uncovered in Niemann-Pick C cells which show lysosomal storage of cholesterol and lack of intracellular cholesterol esterification.
2. Continued studies of cholesterol metabolism and transport in Niemann-Pick C and cystinotic fibroblasts.
3. Characterization of cystinotic cell protein associated with cystine and present in a 2-fold excess in cystinotic versus normal fibroblasts.
4. Investigation of alternative ways of depleting cystine levels in cystinotic fibroblasts.
5. Investigation of metabolism of ascorbic acid in normal and cystinotic fibroblasts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 00408-07 HGB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT (*80 characters or less. Title must fit on one line between the borders.*)
Pathophysiology and Treatment of Human Genetic Diseases

PRINCIPAL INVESTIGATOR (*List other professional personnel below the Principal Investigator*) (*Name, title, laboratory, and institute affiliation*)

| | | | |
|---|---|---|---|
| P.I.: | Joan C. Marini | Senior Staff Fellow | HGB, NICHD |
| Others: | Jane D. Filie | NSRA Fellow | HGB, NICHD |
| | Mary Beth Lewis-Carey | Adjunct Technician | HGB, NICHD |
| | Bonnie M. Orrison | Biologist | HGB, NICHD |

COOPERATING UNITS (*if any*)
Department of Rehabilitation Medicine, CC (N.L. Gerber); Laboratory of Developmental Endocrinology, NICHD (G. Chrousos); Section on Metabolism and Mass Spectroscopy, NICHD (A. Yergy).

LAB/BRANCH
Human Genetics Branch

SECTION
Unit on Connective Tissue Disorders

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.8 | 2.0 | 1.8 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (*Use standard unreduced type. Do not exceed the space provided.*)

We have continued our studies to elucidate the molecular basis of heritable disorders of connective tissue disease and to apply this information to the treatment of these disorders. Using the antisense riboprobe system, we have detected mismatches in the type I collagen mRNA of three cases of osteogenesis imperfecta (OI) and are now engaged in isolating the mutant alleles for sequencing: a case of lethal OI with a mismatch in the COOH end of alpha 2(I), type III OI with a mismatch in the middle of alpha I(I), and type IV OI with mismatches in the 5'-end of alpha I(I) and the 3'-end of alpha 2(I).

We are extending our ability to detect mismatches by the implementation of chemical cleavage methodology and by using both sense and antisense riboprobes to screen both strands of PCR-amplified cDNA. We are applying these techniques especially to those cases in which there is evidence of mosaicism, compound heterozygosity or variability of expression.

We have begun to develop a riboprobe system and protein isolation techniques for type III collagen. The combination of systems to detect point mutations in types I and III collagen is aimed at molecular studies of patients with various forms of Ehlers-Danlos (ED) syndrome. We have analyzed the protein synthesized by cultured fibroblasts in five cases of ED and have detected electrophoretic abnormalities in the type I collagen in three cases; the mRNA of these cases is now being screened by the riboprobe technology.

In clinical protocols, we have continued (1) our investigation of the growth deficiency in OI and the responsiveness of OI bone to growth stimulation, and (2) the physical rehabilitation and bracing protocol for children with moderately severe OI. We have also initiated a collaborative investigation of the dynamics of skeletal calcium in OI children.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00410-05 HGB |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Metabolism in Children with Glycogen Storage Disease (GSD), Type I

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: James B. Sidbury Adjunct Scientist HGB, NICHD

COOPERATING UNITS *(if any)*
Laboratory of Theoretical and Physical Biology, NICHD (N. Esteban and A.L. Yergey).

LAB/BRANCH
Human Genetics Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | 0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

COMBINED WITH Z01 HD 00133-13 HGB

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00412-03 HGB |
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PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Regulation of Gene Expression

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | Richard J. Maraia | Senior Staff Fellow | HGB, NICHD |
| Others: | Samuel Adeniyi-Jones | Expert | HGB, NICHD |
| | Susan Adeniyi-Jones | Adjunct Scientist | HGB, NICHD |
| | Christina Puchalski | Adjunct Scientist | HGB, NICHD |

COOPERATING UNITS *(if any)*

Laboratory of Molecular Biology, NIDDK (Alan Wolffe).

LAB/BRANCH
Human Genetics Branch

SECTION
Unit on Molecular Biology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.75 | 1.75 | 0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The unit has studied the expression of short interspersed repetitive sequence genes of unknown function, into RNA and ribonucleoprotein particles (RNP).

1. We have pursued structural studies of various B1-Alu RNAs. A method was developed to screen genbank for B1 sequences that have the potential to form secondary structures equivalent to the Alu domain found in both the SRP and AFP-derived B1 RNAs. In vivo expressional analysis of some of these sequences has shed light on requirements for in vivo processing of B1-Alu primary transcripts. In order to perform experimental determinations of B1-RNA structure, a recombinant plasmid was constructed which allows T7 polymerase-directed in vitro synthesis of RNA corresponding precisely to either the 210 nt primary or 135 nt processed AFP-derived species. This construct can easily be modified to accommodate other naturally occurring B1 genes.
2. A method was developed for the selective isolation and cloning of B1 sequences expressed as small cytoplasmic RNAs in murine tissues and cell lines. The method involved synthesis of cDNA from gel purified small cytoplasmic B1 RNA followed by homopolymeric tailing and PCR amplification. Subcloning into a derivative of the T7 promoter-containing plasmid described above allows the production of RNA which corresponds to naturally occurring small cytoplasmic RNA. This can be used for sequence and structure analysis, and identification of motifs important for proper expression.
3. The B1-Alu RNA previously identified in murine hepatoma cells which is homologous to the processed 135 nt B1 RNA made in microinjected Xenopus oocytes, has been further characterized. Sedimentation and biochemical characteristics provided further insights into the possible role of this RNA in cellular metabolism.
4. The distribution of the polypeptide Be (p63) previously found to be associated with B1-RNA in microinjected oocytes has been extended to Xenopus Tissue Culture (XTC). As was previously found in Hela cells, synthesis of Be was detectable only after significant cellular proliferation. In XTC Be appeared only after synchronized proliferation of previously quiescent cells, indicating that this protein is either stable with low turnover, or expressed in a cell cycle dependent fashion.
5. Construction of plasmids containing 5' deletions of the AFP-derived B1 gene and no RNA pol II promoters has allowed the continued investigation of B1-specific transcription by Pol III.

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|---|---|
| **DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00413-01 HGB |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Effect of Dichloracetate in Type Ia Glycogenosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | James B. Sidbury | Adjunct Scientist | HGB, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Human Genetics Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NICHD, NIH Bethesda, MD 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.2 | 0.2 | 0 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

COMBINED WITH Z01 HD 00133-13 HGB

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00414-01 HGB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Neurogenetics and Neural Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I. | Lawrence R. Charnas | Senior Staff Fellow | HGB, NICHD |
| Others: | Walter K. Kraft | Biologist | HGB, NICHD |
| | Stephen A. Scott | Junior Fellow | HGB, NICHD |
| | Lee A. Snyder | Summer IRTA | HGB, NICHD |

COOPERATING UNITS *(if any)* Department of Rehabilitation Medicine, CC (U.A. Chaudhry); Medical Neurology Branch, NINDS (M. Dalakas, J. Grafman); OCD, NINDS (R. Gilliatt); Clinical Branch, NEI (M. Kaiser-Kupfer, M. Datilles); Lab. of Neurochem., NINDS (H. Gainer); Molecular Disease Branch, NHLBI (J. Hoeg, D. Rader); Department of Clinical Pathology, CC (N. Papadopoulos).

LAB/BRANCH

Human Genetics Branch

SECTION

Unit on Neurogenetics

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.7 | 1.0 | 0.7 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Clinical Studies:
a) The clinical phenotype in patients with and female carriers of the oculocerebrorenal syndrome of Lowe (OCRL) has been more precisely defined. Patients present a variety of specific ocular, renal, bone, central nervous system, muscle, and soft tissue complications. Heterozygote complications are predominantly limited to the ocular lens, but some degree of renal and central nervous system complications may be present. b) Neurologic manifestations of nephropathic cystinosis, hereditary hemorrhagic telangiectasia, and von Hippel-Lindau disease, genetic diseases that do not present with neurologic complications, have been described. c) The lack of specificity for brain anatomic lesions leading to self injurious behavior in retarded children has been demonstrated.

Laboratory Studies:
a) Elevation of nucleotide pyrophosphatase levels in OCRL cells is not a specific cellular phenotype for OCRL and is unlikely to be useful in attempts at isolating the gene for OCRL. b) In an amphibian system, we have isolated and sequenced full length cDNA clones for XNIF, a neuronal intermediate filament with unusual amino acid sequence, genomic organization, developmental expression profile, and adult neuron specificity, and isolated multiple clones for Xenopus low molecular weight neurofilament protein, (XNF-L).

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00909-11 HGB |

PERIOD COVERED
October 1, 1989 to August 1, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Fetal Alcohol Syndrome

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.: Anil B. Mukherjee Head HGB, NICHD

COOPERATING UNITS *(if any)*

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Developmental Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS:<br>0 | PROFESSIONAL:<br>0 | OTHER:<br>0 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

TERMINATED

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00910-11 HGB |
|---|---|

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biochemistry, Molecular Biology and Physiology of Phospholipase A2 Inhibitory Proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | Anil B. Mukherjee | Head | HGB, NICHD |
| Others: | Norman Dubin | IPA | HGB, NICHD |
| | Lucio Miele | Visiting Associate | HGB, NICHD |
| | Eleonora Cordella-Miele | Visiting Fellow | HGB, NICHD |
| | Lalita Murty | Biologist | HGB, NICHD |
| | Andrew Chen | Summer IRTA Fellow | HGB, NICHD |
| | Giuditta Mantile | Visiting Fellow | HGB, NICHD |

COOPERATING UNITS *(if any)*
GWU (M. Manyak), U. of Florida (M. Lim), Johns Hopkins (H. Zacur), Georgetown U. (R. Dhanireddy), Naval Research Lab (K. Ward, N. Pattabiraman), NEI (C. Chan), Walter Reed Army Med. Center (S. Chock), NIAMAS (R. Wilder, L. Crofford), U. of Pittsburgh (Drs. Singh & Katyal).

LAB/BRANCH
Human Genetics Branch

SECTION
Section on Developmental Genetics

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 4.5 | 4.0 | 0.5 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

During the past year, we have discovered a novel post-translational modification of PLA2 by transglutaminase (TG) which dramatically increases phospholipase A2 (PLA2) activity. TG-catalyzed incorporation of polyamine substrates of TG into PLA2 also significantly increases PLA2 activity. This post-translational modification leads to dimerization of PLA2 which is essential for the activation of this enzyme. Whether this form of post-translational modification of PLA2 plays a role in the activation of this enzyme, found in juvenile rheumatoid arthritis (JRA) and rheumatoid arthritis (RA), is now being investigated. The results of a follow up study on the mechanism of inhibition of PLA2 activity by uteroglobin (UG) and antiflammin peptides suggest that these inhibitors directly interact with PLA2 and inhibits the activation of this enzyme, possibly by interfering with the dimerization process. Using an animal model of human anterior uveitis we have demonstrated that topical application of antiflammin peptides is just as effective as corticosteroid therapy for this disease and are seemingly devoid of the side effects of steroid treatment. A collaborative clinical trial of these peptides on human anterior uveitis is now being implemented. In a separate study, we developed a monospecific neutralizing PLA2 antibody which dramatically inhibits the activity of this enzyme. Purified IgG, Fab2 and Fab1 molecules are equally effective as PLA2 inhibitors. Development of specific PLA2 inhibitory oligopeptides on the basis of this antibody structure is now underway. A cDNA coding for the human UG-like protein has been obtained and now being expressed in E. coli using an expression vector pLE 103-1 which we developed in our laboratory. Characterization and site-directed mutagenesis studies are conducted to determine the structure-function relationship of this protein. In a separate study, we compared the pattern of arachidonate release in bradykinin (BDK)-stimulated fibroblasts from Cystic Fibrosis (CF) patients and those obtained from healthy individuals. We found that although there is no difference in the basal level of arachidonate release between normal and CF cells there is a significant difference among these cells after stimulation with BDK. The relationship between CFTR mutation and abnormal arachidonate release as they relate to chloride channel abnormality in CF is now being investigated.

| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00912-11 HGB |
|---|---|

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Gene Regulation and Cellular Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| P.I.: | Janice Y. Chou | Head | HGB, NICHD |
| Others: | Ke-Jian Lei | Visiting Scientist | HGB, NICHD |
| | Takeshi Watanabe | Visiting Fellow | HGB, NICHD |
| | Leslie Shelly | Visiting Fellow | HGB, NICHD |
| | Cathie Plouzek | NRC Biotechnology Fellow | HGB, NICHD |
| | Er-Li Wang | Courtesy Associate | HGB, NICHD |

See Attached

COOPERATING UNITS *(if any)* Purdue University, IN (Drs. I. Sun and F.L. Crane); Universitats-Krankenhaus, Federal Republic of Germany, (Dr. W. Hopper); Inst. of Cell and Tumor Biology, German Cancer Research Center, Federal Republic of Germany (Drs. G. Schutz and S. Ruppert); University of Florida (Dr. D. Chen); University of Colorado Health Sciences Center, CO (Dr. K.K. Leslie).

LAB/BRANCH
Human Genetics Branch

SECTION
Cellular Differentiation Section

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 8.5 | 6.0 | 2.5 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We conducted studies on the regulation of gene expression in placenta and liver during normal and abnormal differentiation processes. To examine expression of the human pregnancy-specific B1-glycoprotein (PSBG) gene in placenta, we isolated and characterized cDNA and genomic clones encoding this protein. PSBG shares strong sequence homology with the carcinoembryonic antigen, is a member of the immunoglobulin superfamily, and is encoded by multiple genes linked on chromosome 19. We demonstrate that the structure of the PSGG1 gene, which encodes the major PSBG transcript, PSG93, consists of 5 exons and its 3' exon contains multiple splice sites resulting in the generation of five PSBG transcripts. We also discovered that one of the PSBG genes, PSGGB, was preferentially expressed in hydatidiform molar tissues. The PSGGB-encoded species contains a deletion of one amino acid in the N protein domain as compared with the PSGG1-encoded species. Moreover, the PSGGB-encoded protein contains the Arg-Gly-Asp tripeptide which has been implicated in cellular adhesion, suggesting a possible function for these PSBG species.

In our placental alkaline phosphatase (AP) project, we examined the structure-function relationship of human germ cell AP using site-directed mutagenesis and expression in COS-1 cells. Enzyme synthesis and catalytic activity of AP mutants that lack one or both of the glycosylation sites were analyzed and our data indicate that the N-linked glycans are not required for the catalytic activity or stability of germ cell AP. By site-directed mutagenesis, we demonstrate that the serine residue at position 92 is the active site of germ cell AP.

In our project on liver differentiation, we examined expression of three liver genes, phosphoenolpyruvate carboxykinase (PEPCK), tyrosine aminotransferase (TAT), and albumin in a temperature-sensitive adult rat hepatocyte line. Expression of all three genes was stimulated by glucocorticoids and cAMP. However, retinoic acid enhances expression of the PEPCK gene but inhibits expression of albumin and TAT genes induced by glucocorticoids or cAMP. The PEPCK gene is mainly regulated at the transcriptional level; in contrast, the TAT gene is regulated at the post-transcriptional level.

| | | | |
|---|---|---|---|
| Others: | Moshe Zilberstein | Adjunct Scientist | HGB, NICHD |
| | Chen Wang | Adjunct Technician | HGB, NICHD |
| | Adam Sartwell | Biology Aid | HGB, NICHD |
| | Xun Luo | Adjunct Technician | HBG, NICHD |

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00610-10 DEB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Growth, Puberty, their Disorders:Physiology, Pathophysiology, and Molecular Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
PI: Gordon B. Cutler, Jr. Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*
(see attached)

LAB/BRANCH
Developmental Endocrinology

SECTION
Section on Developmental Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 9.5 | 8.0 | 1.5 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to advance understanding of the mechanisms that underlie normal and abnormal puberty, and to apply this knowledge to improve existing therapy for disorders of puberty. Since somatic growth is a major determinant of the timing of pubertal onset, a further objective is to clarify the mechanisms of normal growth and of growth failure. Principal areas of clinical investigation include the mechanism of premature thelarche and of the gonadotropin-independent forms of precocious puberty, the developmental changes in hypothalamic regulation of gonadotropin secretion, the behavioral changes associated with normal and abnormal pubertal development, the mechanisms of prepubertal and pubertal growth, the role of pubertal sex steroids in the acquisition of normal adult bone density, the treatment of central precocious puberty with an analog of luteinizing hormone-releasing hormone, the treatment of familial male isosexual precocious puberty with combined antiandrogen and aromatase inhibitor, the evaluation of new approaches to the diagnosis of growth hormone deficiency and to the differential diagnosis of delayed puberty, and the treatment with growth hormone of children with Turner syndrome and with non-growth hormone-deficient short stature.

The principal areas of laboratory investigation include the structure and function of the regulatory sequences of the human gonadotropin-releasing hormone (GnRH) gene, the structure of the human GnRH gene in families with GnRH-dependent precocious puberty or with idiopathic hypogonadotropic hypogonadism, linkage analysis of the gene for familial male precocious puberty to the luteinizing hormone receptor or other loci, and hormonal regulation of epiphyseal transforming growth factor-$\beta$, fibroblast growth factor, and platelet-derived growth factor. To examine the effects of these growth factors in vivo we are studying rabbits bearing small needles permanently implanted into the proximal tibial epiphyses and connected to osmotic minipumps.

| | | | |
|---|---|---|---|
| Others: | D.L. Loriaux | Head, SSH | DEB, NICHD |
| | B. Albertson | Adjunct Scientist | DEB, NICHD |
| | K.M. Barnes | Chemist (Tech) | DEB, NICHD |
| | J. Baron | Medical Officer | DEB, NICHD |
| | F. Cassorla | Head, ULGP | DEB, NICHD |
| | C. Chan | Summer Pro. (FAES) | DEB, NICHD |
| | D. Counts | Staff Fellow | DEB, NICHD |
| | H. Dichek | Med. Staff Fellow | DEB, NICHD |
| | A. Fahrenkopf | Summer Pro. (pre-IRTA) | DEB, NICHD |
| | P. Feuillan | Adjunct Scientist | DEB, NICHD |
| | Z. Huang | Visiting Fellow | DEB, NICHD |
| | G. Kamp | Adjunct Scientist | DEB, NICHD |
| | L. Laue | Adj. Sci. (IPA-G'town) | DEB, NICHD |
| | S. Malozowski | Adjunct Scientist | DEB, NICHD |
| | G. Marin | Visiting Fellow | DEB, NICHD |
| | G. Municchi | Adjunct Scientist | DEB, NCIHD |
| | Y. Nakayama | Adjunct Scientist | DEB, NICHD |
| | J. Novosad | Adjunct Sci. (Int. NRSA) | DEB, NICHD |
| | K. Oerter | Med. Staff Fellow | DEB, NICHD |
| | S. Rose | Adjunct Scientist | DEB, NICHD |
| | J. Levine Ross | Adjunct Scientist | DEB, NICHD |
| | A. Sobel | Summer Pro. (pre-IRTA) | DEB, NICHD |
| | P. Thomas | Med. Staff Fellow | DEB, NICHD |
| | J. Yanovski | Med. Staff Fellow | DEB, NICHD |

Cooperating Units

LDP, National Institute of Mental Health (E. Susman, E. Nottelmann, G. Inoff, L. Dorn); Clinical Center, NIH (M. Royster, J. Jones, L. Long, G. Heavner, S. Hill, A. Dwyer, T. Shawker; MCNEB, National Institute of Diabetes, Digestive, and Kidney Diseases, (Yuko Nakayama, B. Weintraub); Department of Pediatrics, University of Indiana (O. Pescovitz); Department of Internal Medicine, McMaster University Medical Center (J. Booth); LMG, NICHD(E. Lee, H. Westphal), Department of Pediatrics, Case Western Reserve University (S. Radovick, F. Wondisford); LNC, National Institute of Neurological Disorders and Stroke (S. Wray); ARB, National Institute of Arthritis and Musculoskeletal and Skin Diseases (M. Bolander, T. Blaylock); LNS, National Institute on Aging (D. Murphy); LMINS, National Institute of Enviromental and Health Sciences (P. Manasco);

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00628 01 DEB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Paracrine and Autocrine Roles in Insulin-like Growth Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | C. Bondy | Head, UNR | DEB, NICHD |
| Others: | J. Zhou | Visiting Fellow | DEB, NICHD |
| | E. Chin | Clinical Associate | DEB, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Unit on Neuroendocrine Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 0.5 | 0.5 | |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Insulin-like growth factors (IGFs) have long been familar as somatomedins, synthesized in and released from the liver in response to growth hormone stimulation, and acting in an endocrine fashion to promote the growth of peripheral somatic tissue. Recent evidence suggests that IGFs may also have autocrine/paracrine roles in specific aspects of the growth and differentiation of the embryo and in the postnatal response of certain organs to injury. We have shown that during early embryonic development in the rat, IGF-1 mRNA is concentrated in target zones of the developing trigeminal and sympathetic nervous systems. IGF receptor mRNA has been localized to the ventral floorplate of the developing hindbrain and spinal cord, a region involved in axonal targeting and establishing the polarity of neural growth. Perinatally, IGF-1 mRNA was localized in maturing neurons of differentiating somatosensory systems. The topographic and temporal pattern of IGF-1 gene expression in these different settings is strongly suggestive of a role for IGF-1 in the development of neural connectivity.

Further studies have shown that IGF-1 and IGF receptor mRNAs are localized in anatomically complementary regions of the adult rat kidney, adrenal and gonad, suggesting a possible role for IGF-1 in physiologic autoregulation of the size and function of these organs in postnatal life. Future directions include the investigation of the role of IGFs in neural development and plasticity and in mature organ hypertrophy with a view toward developing therapeutic applications for IGFs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00631-01

PERIOD COVERED

October 1, 1989 to September 30 ,1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Neuroendocrine Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | G. Aguilera | Head, SEP | DEB, NICHD |
| Others: | M. Familari | Visiting Fellow | DEB, NICHD |

COOPERATING UNITS *(if any)*

Dept. of Psychiatry, UCSD (R.L. Hauger)
Dept. of Pharmacology, Howard University (Y. Tizabi)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Endocrine Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.2 | 1.2 | |

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Investigation has focused in the mechanisms of interaction between corticosterone releasing hormone (CRH) and other regulators in the control of the hypothalamic-pituitary-adrenal axis in normal conditions and during adaptation to stress.

Studies during aging in two strains of rats which differ in their responsiveness to stress, showed reduced concentration of pituitary CRH receptors in old rats. Despite the decrease in receptors in both strains, basal and stress stimulated ACTH were comparable to those in young rats. However, stress induced corticosterone responses were more prolonged in old rats. These data suggest that a defect in adrenal responsiveness to ACTH may have an important role in the alterations of the hypothalamic-pituitary adrenal axis during aging.
Previous studies showed that sustained stress is accompanied by decreases in pituitary CRH receptors and pituitary densensitization to the primary stress, but a hypersensitivity to a novel stress. The role of CRH and VP on these changes were studied by analysis of plasma ACTH and corticosterone responses to acute stress in rats receiving chronic minipump infusions of CRH alone or in combination with VP. CRH and CRH plus VP infusion caused the expected decrease in CRH receptors, but in contrast with the changes in chronic stress, ACTH responses to acute stress were markedly reduced in rats receiving the infusions. Plasma ACTH responses to acute CRH or CRH plus VP injection, as well as releasable ACTH pools measured in vitro by stimulation with 40 mM KCL were also reduced following chronic CRH treatment. The data indicate that sustained pituitary account for the enhanced ACTH responses to a novel stimulus during chronic stress.

Studies in forebrain neuronal cell cultures showed that CRH stimulates cyclic AMP production. Similar to the pituitary, this effect was enhanced by the protein kinase C stimulators VP and phorbol esters, and inhibited by glucocorticoids. The data supports a role for CRH in the regulation of extrahypothalamic neuronal function.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00623-07 DEB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Adrenal Physiology, Pathophysiology, and Molecular Biology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Gordon B. Cutler, Jr. Head DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

See attached list

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Section on Developmental Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS:<br>3.2 | PROFESSIONAL:<br>3.1 | OTHER:<br>0.1 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We seek to advance understanding of the mechanisms that cause adrenal androgen secretion by the fetal adrenal zone prenatally and by the definitive adrenal cortex during adrenarche, and to improve the diagnosis and treatment of disorders that cause excess adrenal androgen or glucocorticoid secretion, such as premature adrenarche, congenital adrenal hyperplasia, adrenal neoplasms, idiopathic hirsutism, polycystic ovary syndrome, and Cushing's syndrome. We also seek to clarify the pathophysiology of primary adrenal insufficiency (Addison's disease) and secondary adrenal insufficiency and to improve the treatment of these conditions.

Children with congenital adrenal hyperplasia are being enrolled into one of four projects. The first examines the effect of several recent advances in treatment on long-term growth and adult height. The second tests the effect of different dose-schedules of hydrocortisone. The third employs a newly available antiandrogen and an inhibitor of androgen-to-estrogen conversion to block the action of excess androgen levels. The fourth examines the hypothesis that supplemental salt intake will reduce the day-to-day variability, and improve the overall control, of androgen secretion.

Patients with Cushing's syndrome are being studied by several new diagnostic methods to determine the relative diagnostic efficiency of these new methods compared to the old. Patients with Cushing's syndrome due to occult ACTH-producing tumors are being treated with an investigational glucocorticoid antagonist. Oncogene expression in corticotropinomas is also under study in an attempt to elucidate the molecular basis of Cushing's disease.

| | | | |
|---|---|---|---|
| Others: | D.L.Loriaux | Chief | SSH, DEB, NICHD |
| | B. Albertson | Adjunct Scientist | Georgetown University |
| | A. Armstrong | Med. Staff Fellow | DEB, NICHD |
| | K.M. Barnes | Chemist (Tech) | DEB, NICHD |
| | F. Cassorla | Head, ULGP | DEB, NICHD |
| | G. Chrousos | Head, UHRF | DEB, NICHD |
| | P. Feuillan | Adjunct Scientist | Eli Lilly and Company |
| | L. Laue | Med. Staff Fellow | DEB, NICHD |
| | L. Nieman | Expert | Roussel-UCLAF |
| | K. Oerter | Clinical Associate | DEB, NICHD |
| | J. Levine Ross | Adjunct Scientist | Hahnemann University |
| | H. Tracer | Medical Staff Fellow | DEB, NICHD |

Cooperating Units

Chief, Radiology, CC, NIH (J. Doppman); Acting Chief, SNB, NINCDS, NIH (E. Oldfield); Staff Radiologist, Radiology, CC, NIH (A.J. Dwyer); Department of Internal Medicine, McMaster University Medical Center (J. Booth); MCNEB, NIDDK (S. Radovick, F. Wondisford).

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00618-09 DEB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physiology and Pathophysiology of the Hypothalamic-Pituitary-Adrenal Axis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

G.P. Chrousos Head, SPE, DEB, NICHD

COOPERATING UNITS *(if any)*

See attached list

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Pediatric Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 8.1 | 6.1 | 2.0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project we seek to advance the understanding of the physiology and pathophysiology of the hypothalamic-pituitary-adrenal axis. The role of stress-related hormones in normal and disease states is being examined, and clinical applications for these hormones are sought. The recent discovery of the structure of corticotropin releasing hormone (CRH) and the development of sensitive assays for measuring stress-related hormones and their receptors have led to rapid progress in this field. Major progress has been made in three areas:

1) Clinical applications of CRH: An ovine CRH (oCRH) stimulation test has been developed that is useful in the differential diagnosis of adrenal insufficiency, Cushing's syndrome, and pseudo-Cushing's states (psychiatric hypercortisolism). The human CRH (hCRH) analog is useful in studying the physiology of the pituitary-adrenal axis. The oCRH stimulation test and measurement of CSF CRH have increased our understanding of the pathophysiology of Cushing's syndrome, melancholic depression, atypical depression, seasonal depression, the premenstrual tension syndrome, anorexia nervosa, the chronic fatigue syndrome and late-onset congenital adrenal hyperplasia.

2) The regulation of the axis by opioids, vasopressin, oxytocin, glucocorticoids, platelet activating factor (PAF), tumor necrosis factor - alpha, cholecystokinin (CCK), neuropeptide Y, benzodiazepine agonists and antagonists and thyroid hormones has been studied in vivo and/or in vitro. Neurotransmitter and feedback regulation of hypothalamic CRH secretion has also been examined in vitro. Third trimester pregnant women and athletes have a hyperfunctional pituitary-adrenal axis in the resting state. Hypothalamic-pituitary-adrenal axis reactivity and personality traits have been correlated in developing adolescents. The hCRH gene 5' regulatory region has been cloned sequenced and its regulation is being studied.

3) Role and actions of glucocorticoids: The effects of glucocorticoids upon the cardiovascular system during surgical stress are merely permissive. Surgical Stress is associated with continuous and pulsatile secretion of ACTH. Glucocorticoid resistance is an autosomal recessive disease associated with normal size glucocorticoid receptor protein that has decreased affinity for glucocorticoids and normal size mRNA. We have found a nonconserved point mutation (Asp --> Val) in the steroid binding domain of the glucocorticoid receptor which leads to a decrease in the affinity of the receptor for glucocorticoids and decreased glucocorticoid effect. The interaction of the glucocorticoid receptor with the heat-shock proteins is studied.

| | | | |
|---|---|---|---|
| Others: | A.Calogero | Adjunct Scientist | DEB, NICHD |
| | G. Cizza | Adjunct Scientist | DEB, NICHD |
| | L. Dorn | Adjunct Scientist | DEB, NICHD |
| | T. Friedman | Medical Staff Fellow | DEB, NICHD |
| | W. Gallucci | Adjunct Technician | DEB, NICHD |
| | T. Gomez | Clinical Associate | DEB, NICHD |
| | D. Hurley | Adjunct Scientist | DEB, NICHD |
| | E. Johnson | Post Doctoral IRTA | DEB, NICHD |
| | T. Kamilaris | Adjunct Scientist | DEB, NICHD |
| | M. Karl | Visiting Fellow | DEB, NICHD |
| | S. Listwak | Adjunct Technician | DEB, NICHD |
| | A. Margioris | Adjunct Scientist | DEB, NICHD |
| | L. Nieman | Expert | DEB, NICHD |
| | D. Rabin | Adjunct Scientist | DEB, NICHD |
| | E. Rorer | Predoctoral IRTA | DEB, NICHD |
| | C. Stratakis | Adjunct Scientist | DEB, NICHD |
| | N. Vamvakopoulos | Expert | DEB, NICHD |

### Cooperating Units

Clinical Neuroendocrinology Branch, National Institute of Mental Health, NIH (P.W. Gold); Surgical Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, NIH (E. Oldfield); Laboratory of Developmental Psychology, National Institute of Mental Health, NIH (E. Nottleman); Human Performance Laboratory, Dept of Military Medicine, USUHS (P. Deuster); Diabetes Branch, NIDDK, NIH (S. Taylor).

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00616-10 DEB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Structure, Function, and Physiology of Glycoprotein Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | B. C. Nisula | Head, SME | DEB, NICHD |
| Others: | D. Blithe | Expert | DEB, NICHD |
| | A. Bennet | Visiting Fellow | DEB, NICHD |
| | M. Flack | Medical Staff Fellow | DEB, NICHD |
| | C. Lyons | Bio. Lab. Tech. | DEB, NICHD |

COOPERATING UNITS *(if any)*

Fondation de Recherche en Hormonologie, Paris. France

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Medical Endocrinology Section

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3.5 | 3.0 | 0.5 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The overall objectives of this project are to understand the endocrinology of the human glycoprotein hormones, thyroid-stimulating hormone (TSH), choriogonadotropin (hCG), luteinizing hormone (LH), and follicle-stimulating hormone (FSH), and thereby to develop diagnostic and therapeutic clinical applications. Recent research advances in the current period include the following: Development if a new diagnostic test, the nocturnal TSH surge test, for diagnosis of central hypothyroidism in children and demonstration of its superiority to the standard thyrotropin-releasing hormone test; elucidation of the structure of the oligosaccharide moieties of the $\beta$-core molecule of pregnancy; delineation of the fundamental kinetic parameters governing $\beta$-core metabolism in human subjects; determination of the $\beta$-core levels in pregnancy serum and in postmenopausal urine; and demonstration of the primary nephrogenous origin of $\beta$-core in normal pregnancy. Future directions of the project will include the following: Investigation of structure-function relationships of the glycoprotein hormones using molecular biological techniques such as site-directed mutagenesis; characterization of the natural evolution of the heterogeneity of oligosaccharide structures of hCG and its subunits throughout pregnancy; and assessment of the roles of defects in the nocturnal TSH surge and subtle abnormalities of pituitary-thyroid axis function as causes of short stature and delayed growth in childhood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

# NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00621-08 DEB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanisms of Linear Growth

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: F. Cassorla Head, UGP DEB, NICHD

Others: (see attached)

COOPERATING UNITS *(if any)*

Metabolism Branch, NCI, NIH (P. Nissley): Department of Pediatrics, Medical College of Pennsylvania (J. Levine-Ross).

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Growth Physiology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.5 | 2.5 | |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☒ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is to investigate the hormonal mechanisms that are responsible for linear growth. Principal areas of investigation include improving the accuracy of the methods employed to diagnose growth hormone deficiency. We are also studying the effects of growth hormone and sex steroid administration on linear growth in patients with Turner's syndrome and delayed puberty. We are determining the cortisol production rate in normal children and adolescents. In addition, we are studying the mechanism of catch up growth in a primate model. We are determining the cortisol production rate and metabolic clearance rate in normal children and adolescents. We are studying the hormonal regulation of transcriptional expression of the growth hormone receptor gene. In addition, we are examining the effect of inducing pubertal delay in children with extreme short stature, in order to prolong prepubertal growth prior to the pubertal spurt and possibly enhance ultimate height by delaying epiphyseal fusion. We are also investigating the effects of growth hormone therapy on the adult height of non-growth-hormone deficient children with short stature through a randomized, double-blind, placebo-controlled clinical trial. In addition, we are investigating the growth hormone secretory dynamics in patients with hypophosphatemic rickets. Finally, we are studying the effects of growth hormone-releasing factor on linear growth in growth hormone-deficient children by using different treatment regimens in order to optimize growth.

| | | | |
|---|---|---|---|
| Others: | G. Marin | Visiting Fellow | DEB, NICHD |
| | A. Cristiano | IRTA Fellow | DEB, NICHD |
| | H. Domene | Exchange Scientist | DEB, NICHD |
| | S. Rose | Adjunct Scientist | DEB, NICHD |
| | G. Municchi | Adjunct Scientist | DEB, NICHD |
| | G. Cutler | Chief, SDE | DEB, NICHD |
| | G. Merriam | Head, URE | DEB, NICHD |

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

## NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HD 00622-08 DEB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diagnostic and Therapeutic Applications of Growth Hormone-Releasing Hormones

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | G. R. Merriam | Head, URE | DEB, NICHD |
| Others: | (see attached) | | |

COOPERATING UNITS *(if any)*

(see attached)

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

Unit on Reproductive Endocrinology

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.6 | 1.6 | 0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Growth hormone (GH), the pituitary hormone which stimulates statural growth and metabolism, is regulated by two hypothalamic peptides: GH-releasing hormone (GHRH) and somatostatin (SRIF). Most patients with GH deficiency (GHD) have a deficiency of GHRH secretion. This project aims to characterize the physiology of GHRH and SRIF regulation and to apply these findings in the diagnosis and therapy of GH deficiency and excess.

Both dopaminergic (DA) and noradrenergic (NE) agents stimulate GH secretion, while $\beta$-adrenergic agents (E) stimulate SRIF; but the detailed regulation of this system is not understood. Monkeys given diethyldithiocarbamate (DDTC), which inhibits conversion of DA to NE, show markedly reduced responses to GHRH and spontaneous GH secretion, which is not reversed with $\beta$-adrenergic blockade. This indicates that DA requires local conversion to NE to stimulate GHRH secretion, and that NE may inhibit secretion of SRIF. Rats given chronic DDTC treatment show reduced growth. This provides a new model of reversible GHD.

Since GHD largely results from deficiency of GHRH, GHRH therapy can treat GHD. We have conducted a cooperative trial of 1 year GHRH therapy in a large group of GHD children. GHRH is fully effective in restoring normal growth velocity in the majority of patients.

GH responses to GHRH are highly variable, due to variable interference by SRIF. We have tested the utility of coadministration of SRIF blockade in combination with GHRH therapy of GHD. Preliminary results suggest that E blockade enhances the growth response to GHRH.

GHRH is a large molecule, but we have found a high degree of GH-releasing activity in a synthetic hexapeptide (GHRP) which is effective orally. GHRP appears to stimulate release of GHRH.

Pituitary tumors hypersecreting GH (acromegaly) retain responsivity to GHRH, although systemic counterregulatory responses (SRIF) are inceased. We have developed a candidate ligand-directed cytotoxic agent for therapy of acromegaly by coupling GHRH to an organoboron compound which undergoes $\alpha$ decay when exposed to neutrons.
Thus GHRH may form the basis of novel approaches to the therapy of GHD and GH excess.

| | | | |
|---|---|---|---|
| OTHERS: | F. Cassorla | Head, UGP | DEB, NICHD |
| | H. C. Chen | Senior Investigator | ERRB, NICHD |
| | A. Cristiano | IRTA Fellow | DEB, NICHD |
| | E. Hao | Visiting Fellow | DEB, NICHD |
| | S. Malozowski | Visiting Scientist | DEB, NICHD |
| | M. Pleti | Adjunct Scientist | DEB, NICHD |

Cooperating Units:

Surgical Neurology Branch, NINDS (E. Oldfield); Dept. of Radiology, Clinical Center (J. Doppman); Clinical Neuroendocrinology Branch, NIMH (E. Mamalaki); State University of New York, Stony Brook (M. Gelato); Peking Union Medical College (E. Shih).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 HD 00625-03 DEB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Neuroendocrine Regulation of Reproductive Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. R. Merriam Head, URE DEB, NICHD

Others: (see attached list)

COOPERATING UNITS *(if any)*
(see attached list)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Unit on Reproductive Endocrinology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 3 | 2.8 | 0.2 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project studies the neuroendocrine control of reproduction in animals and human subjects, with focus on a) the regulation of the ovulatory cycle and episodic gonadotropin secretion; b) photoperiodic control of seasonal reproduction; c) hypothalamic and lactational amenorrhea; d) premature ovarian failure; and e) luteal phase dysphoric disorder:

(A) Ovulatory Cycle: The role of progesterone in the regulation of ovulation was studied in cycling women and in rats. In women, blockade of the preovulatory rise in progesterone consistently delayed ovulation, an effect reversed with progesterone administration, indicating an important role for progesterone in the timing of ovulation. In rats, mRNA's for hypothalamic CRH and pro-opiomelanocortin (POMC) showed marked variations around the time of the ovulatory gonadotropin surge, suggesting that in opiate peptides may also modulate the surge. POMC changes paralleled those of levels of progesterone.

(B) Photoperiodism: Previous work has established that photoperiod strongly modulates reproductive function in rhesus monkeys. Pinealectomy abolishes these responses and leaves animals either acyclic or with free-running cycles.

(C) Lactational Amenorrhea: Lactating amenorrheic women were treated with pulsatile gonadotropin-releasing hormone. This regimen restored cyclic ovarian function to a degree identical to that of control subjects, indicating that the interruption of the menstrual cycle during lactation is entirely central. Because opioids can underlie hypothalamic amenorrhea, CRH and POMC mRNA's were studied in lactating anestrus rats. Both peptide messages were markedly reduced.

(D) Premature Ovarian Failure (POF): was studied in patients and in a mouse model of autoimmune ovarian disease. In contrast to normal menopause, many patients with POF were found to have follicles, which allows the possibility of remission. In the A/J mouse strain, neonatal thymectomy leads to failure of clonal deletion with autoimmune ovarian failure; however, other strains do not manifest disease after thymectomy, indicating alternative mechanisms of tolerance.

(E) LLPDD: The linkage between symptoms and the luteal phase of the cycle was studied by truncating the luteal phase with a progesterone antagonist. Despite the hormonal changes, symptoms followed the same time course as in the control groups. This finding suggests a re-evaluation of the biology of LLPDD as an intrinsically periodic disorder for which the cycle serves as a Zeitgeber.

| Others: | | | |
|---|---|---|---|
| | A.Y. Armstrong | Adjunct Scientist | DEB, NICHD |
| | M.C. Batista | Visiting Fellow | DEB, NICHD |
| | H.C. Bohler, Jr. | NRSA Fellow | DEB, NICHD |
| | L. Liu | Adjunct Scientist | DEB, NICHD |
| | N. Ma | Adjunct Scientist | DEB, NICHD |
| | L.M. Nelson | NRSA Fellow | DEB, NICHD |
| | L.K. Nieman | Expert | DEB, NICHD |
| | M. Zinaman | IRTA | DEB, NCIHD |

Cooperating units:

NIDDK, NIH (Dr. B. White); NCI (Dr. A. Kruisbeek); Clinical Immunology, CC, NIH (Dr. T. Fleisher); Clinical Psychobiology Branch, NIMH (D. Rubinow, P. Schmidt); Georgetown University Medical School (E. Libre, J. Zawadski), Dept. of Physiology, University of Alberta (C. Chik); Dept. of Statistics, Univ. of California, Berkeley (K.W. Wachter); Dept. of Anatomy, Univ. of Missouri (S. Petersen, T. Zoeller).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00626-02 DEB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Progesterone Action in Reproduction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | L.K. Nieman | Expert | DEB, NICHD |
| Others: | M. Batista | Visiting Fellow | DEB, NICHD |
| | J. McCarthy | MSF | DEB, NICHD |

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 1.8 | PROFESSIONAL: 1.8 | OTHER: 0 |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Progesterone is essential for the normal morphologic development of the luteal phase endometrium, for implantation of a blastocyst and for maintenance of pregnancy. Surprisingly, given its critical role in reproduction, little is known about the mediator(s) of progesterone action. We are using a recently described progestin-dependent glycoprotein, placental protein 14 (PP14) and the antiprogestin RU 486 to probe progesterone action. We are investigating the relationship between endogenous progesterone production, serum PP14 concentrations and endometrial maturation in women with normal cycles and those with luteal phase defects. We are also examining the secretion of PP14 by endometrial explants obtained from normal women, those with idiopathic infertility and those with habitual abortion. These studies suggest that serum PP14 concentrations and direct examination of endometrial secretion of PP14 may be better indicators of uterine function than those currently employed. Further studies in infertile women and those with multiple miscarriages may give better insight into the cause, and possibly, the treatment of these disorders.

Guinea pigs, like women, require progesterone for normal endometrial development, implantation and pregnancy. We have used the antiprogestin RU 486 to probe the process of implantation in this animal model. Small daily doses of the drug result in a marked decrease in implantation sites, confirming the critical role of progesterone in nidation. Studies in non-pregnant women show that small daily doses of RU 486 delay the normal maturation of the endometrium without significantly changing the hormonal patterns or timing of the menstrual cycle. Serum PP14 concentrations were significantly decreased in the women receiving RU 486, showing that progesterone (and not some other product of the corpus luteum) is critical for normal endometrial morphology. No adverse effects were seen in either study. Taken together, these data suggest that RU 486 has potential as a contraceptive agent.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00630-01 DEB

PERIOD COVERED

October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hypercortisolism: Diagnosis and Treatment

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | L.K. Nieman | Expert | DEB, NICHD |
| Other: | M. Flack | MSF | DEB, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH

Developmental Endocrinology Branch

SECTION

INSTITUTE AND LOCATION

NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1.0 | 1.0 | |

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cushing's syndrome, a fatal disease, is suspected in many thousands of patients each year, but confirmed in only a fraction of these. There is no test that reliably separates individuals with pseudo-Cushing's states, who have mild hypercortisolism and minimal physical features of Cushing's syndrome, from those with mild or intermittent Cushing's syndrome, who may present in an identical fashion. We are exploiting the different pathophysiologies in the pseudo-Cushing's and Cushing's syndrome states to develop new tests for making this distinction. Since the hypercortisolism of patients with pseudo-Cushing's syndrome reflects hypersecretion of CRH, we are examining whether dexamethasone, a potent glucocorticoid, can suppress CRH production and blunt the ACTH and cortisol responses to exogenous ovine CRH in these patients. We find that a screening UFC measurement may be normal in 5% of patients with Cushing's syndrome. Midnight cortisol levels, in contrast, appear to better discriminate the two groups. Studies with oCRH have revealed similar hormonal responses in Cushing's disease and pseudo-Cushing's syndrome; interestingly, dexamethasone pre-treatment significantly reduces the responses in pseudo-Cushing's patients. Further work will explore whether inferior petrosal sinus sampling for ACTH and CRH levels with and without dexamethasone can distinguish between normal individuals, those with pseudo-Cushing's syndrome, and those with Cushing's syndrome.

Our second initiative is to improve the approach to the differential diagnosis and treatment of the causes of Cushing's syndrome. We are characterizing the performance of the classic (standard dexamethasone suppression and metyrapone stimulation) and the newer (inferior petrosal sinus sampling, oCRH) differential diagnostic tests. Studies in 243 patients show that inferior petrosal sinus sampling has 100% sensitivity and specificity; a convenient protocol for a morning oCRH stimulation test developed in 101 patients has 100% specificity and 94% sensitivity. Re-evaluation of the standard dexamethasone suppression test has improved its specificity (100%), and retained good sensitivity (83%). The localization of ectopic-ACTH producing tumors continues to be elusive, with only 50% identified initially, despite the application of MR and CT imaging and venous sampling. This problem may yield to ongoing studies using novel ACTH secretagogues as adjuncts to venous sampling. Surgical treatment of Cushing's syndrome remains the treatment of choice, even in patients with recurrent Cushing's disease.

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| DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE<br>**NOTICE OF INTRAMURAL RESEARCH PROJECT** | PROJECT NUMBER<br>Z01 HD 00629-01 DEB |

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Polyol Pathways in Health and Disease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | C. Bondy | Head, UNR | DEB, NICHD |
| Others: | J. Zhou | Visiting Fellow | DEB, NICHD |
| | E. Chin | Clinical Associate | DEB, NICHD |

COOPERATING UNITS *(if any)*
Laboratory of Kidney and Electrolyte Metabolism, NHLBI (B. G. Crowley): Laboratory of Molecular Pharmacology, NEI (P. Kador).

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Unit on Neuroendocrine Regulation

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 1 | 1 | |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Polyhydric alcohols, or polyols, are osmotically active organic molecules whose physiologic and pathophysiologic significance has only very recently come under investigation. These molecules are produced by the reduction of common sugars via the catalyic action of aldose reductase. It appears that the overproduction and accumulation of polyols such as sorbitol, the product of glucose reduction, in certain tissues, may be responsible for some of the dire consequences of chronic hyperglycemia, as in diabetes mellitus. Polyol accumulation in lens, retina and nerve cells has been associated with osmotic injury and development of cataracts, retinopathy and neuropathy. We have described the cellular localization of aldose reductase mRNA in the rat lens epithelium, retinal Meuller cells, peripheral Schwann cells, ovarian granulosa cells and renal inner medullary epithelium. We have shown that aldose reductase gene expression is regulated by local osmotic pressure, and by end-product accumulation in cells of the renal medulla. We have proposed that the function of aldose reductase catalyzed sorbitol production in the renal medulla is to provide an osmotic buffering system for the protection of medullary epithelial cells against the extremely high and varying ambient osmotic stresses of the renal medulla. We have also hypothesized, as a result of finding extremely high levels of aldose reductase mRNA in developing lens epithelial cells, that aldose reductase-catalyzed sorbitol production may drive the huge volume expansion which is the key feature of lens cell differentiation. Future studies will include investigation of the role of aldose and its sister enzyme, aldehyde reductase in normal physiology and in the pathophysiology of diabetes and hereditary galactosemia, focusing on tissues such as kidney, peripheral nerve and ovary, which are primary targets of injury in these diseases. We also will pursue the more basic issue of the phenomenon of osmoregulation of gene expression, using aldose reductase as a model system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00627-01 DEB

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Glycoprotein Hormones: Oligosaccharide Structure and Function

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | D. L. Blithe | Expert | DEB, NICHD |
| Others: | M. Skaraulis | Clinical Associate | DEB, NICHD |
| | C. Lyons | Bio. Lab. Tech. | DEB, NICHD |
| | B. Nisula | Acting Chief | DEB, NICHD |

COOPERATING UNITS *(if any)*
Department of Obstetrics and Gyneocology, University of Pennsylvania Medical Center Philadelphia, Pennsylvania (J. F. Strauss); Department of Medicine, St. Luke's Roosevelt Hospital Center, New York, New York (W. Rosner)

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Medical Endocrinology Branch

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.0 | 1.5 | 0.5 |

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The goal of the project is to increase our understanding of the structure-function relationships of oligosaccharide moieties on glycoprotein hormones and related molecules. The molecules under investigation are human chorionic gonadotropin (hCG), free $\alpha$ molecules associated with pregnancy or malignancy, hCG-$\beta$ subunit, $\beta$-core fragment and corticosteroid binding globulin (CBG). Carbohydrate modifications, resulting in a variety of branched oligosaccharide structures, occur on all glycoproteins prior to secretion. These modifications can effect virtually every aspect of the molecule's behavior, including receptor binding and signal transduction, yet the underlying regulatory mechanisms remain unclear. We have investigated changes in the oligosaccharide moieties as a function of the following parameters:

1. Synthetic environment. Interaction of subunits can either promote or restrict modifications on the oligosaccharide structures produced in the final secreted protein. When hCG-$\beta$ subunit combines with hCG-$\alpha$ subunit prior to carbohydrate processing, hCG-$\beta$ prevents addition of some branches to hCG-$\alpha$, whereas the uncombined (free) $\alpha$ molecules receive the additional branches. The presence of these branched structures on free $\alpha$ prevent it from subsequently combining with $\beta$ to form intact hormone.
2. Gestational development. Glycosylation changes as a function of gestational age. Molecules produced in late pregnancy are more highly branched than those of early pregnancy.
3. Cellular differentiation. Oligosaccharide changes occur in placental cytotrophoblasts as they begin to undergo cellular differentiation.
4. Species specificity. We showed that rat CBG contained a carbohydrate composition strikingly different from that of human CBG. Variations in glycosylation may account for differences in receptor-hormone interactions observed between species.
5. Cellular catabolism. $\beta$-core fragment is derived from hCG-$\beta$ subunit and is the most abundant hCG-related component found in pregnancy urine. $\beta$-core has important potential as a clinical marker for a number of pathologic conditions, including a variety of malignancies. We have characterized the carbohydrate structures on $\beta$-core and compared them to hCG-$\beta$.

The structural analyses described herein further our knowledge about the complexities associated with glycosylation. We plan to probe how the differences observed in glycosylation effect the bioactivity of these molecules.

**DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE**

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER

Z01 HD 00632-01

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Renin-Angiotensin System and Aldosterone Regulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | G. Aguilera | Head, SEP | DEB, NICHD |
| Others: | M.A. Millan | Sr. Staff Fellow | DEB, NICHD |
| | S. Rocco | Visiting Fellow | DEB, NICHD |
| | C. Johnson | Research Associate | DEB, NICHD |
| | P. Feuillan | Research Associate | DEB, NICHD |

COOPERATING UNITS *(if any)*

Contract for preparation of adrenal and pituitary cells N01-HD-0-2806

LAB/BRANCH
Developmental Endocrinology Branch

SECTION
Section on Endocrine Physiology

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, Maryland 20892

| TOTAL MAN-YEARS: | PROFESSIONAL: | OTHER: |
|---|---|---|
| 2.75 | 2.5 | 0.25 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to analyze physiological and pathological aspects of the renin-angiotensin system, including the effects of AII in circulatory homeostasis, fetal development pituitary and gonadal function. AII mediates the increase in aldosterone secretion during sodium restriction, but the adrenal effects of the peptide are dependent on other factors which modulate the sensitivity of the glomerulosa zone to AII.

A novel finding was that serotonin, a known direct stimulator of aldosterone secretion has marked potentiating effect on the steroidogenic actions of AII. In contrast with the direct stimulatory effect of 5HT on aldosterone production which is mediated by cAMP, the enhancement of the effect of AII is due to potentiation of AII induced increases in cytosolic calcium.

The interaction between a AMP dependent and independent mechanisms in the regulation of aldosterone secretion was further studied in isolated adrenal glomerulosa cells. Activation of protein kinase C (PKC) by preincubation of the cells with phorbolesters (PBE) results in about 30% decrease in ACTH stimulated aldosterone and cAMP production, while PKC inhibition caused enhancement of the ACTH responses. In contrast to the effect of PBE preincubation simultaneous addition of ACTH and PBE resulted in potentiation of the ACTH response. The data demonstrates that the modulatory effects of PKC on steroidogenesis is dependent on receptor occupancy, and may be important in the control of the adrenal responsiveness to regulators.

Studies on the central activity of AII were extended to the analysis of brain AII receptors during development. Receptors were present in areas related with water intake and control of blood pressure at all ages. In addition there were dynamic changes in a number of brain areas suggesting that AII has a role in neuronal maturation during development.

A new observation was the demonstration of abundant AII receptors in skeletal muscle and connective tissue of monkey and human fetus, suggesting a role for AII in fetal development in primates. The effects of AII on cellular growth were studied in cultured mesangial cells. AII receptors were present in cultured fetal and adult mesangial cells and incubation of the cells with AII resulted in stimulation of thymidine incorporation and cell division. The data suggests a role for AII in the proliferative response during glomerular disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

**NOTICE OF INTRAMURAL RESEARCH PROJECT**

PROJECT NUMBER
Z01 HD 08719-10 LMGR

PERIOD COVERED
October 1, 1989 to September 30, 1990

TITLE OF PROJECT *(80 characters or less Title must fit on one line between the borders.)*
Development and Uses of Eukaryotic Vectors

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator ) (Name, title, laboratory, and institute affiliation)*

| | | | |
|---|---|---|---|
| PI: | Bruce H. Howard | Chief, Lab.of Molecular Growth Regulation, IRP,NICHD | |
| Other: | A. Giordano | Biotechnology Fellow | LMB, DCBDC,NCI |
| | D. Jones | Biotechnology Fellow | LMB, DCBDC,NCI |
| | T. Howard | Chemist | LMGR, NICHD |
| | R. Vorce | PRAT Fellow | LMGR, NICHD |
| | K. Sakamoto | Visiting Associate | LMGR, NICHD |
| | Y. Okada | Guest Researcher | LMGR, NICHD |
| | D-Y. Chang | Biotechnology Fellow | LMGR, NICHD |
| | R. Maraia | Senior Staff Fellow | LMGR, NICHD |
| | C. Driscoll | Biologist | LMGR, NICHD |

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Growth Regulation

SECTION

INSTITUTE AND LOCATION
NICHD, NIH, Bethesda, MD 20892

| | | |
|---|---|---|
| TOTAL MAN-YEARS: 6.3 | PROFESSIONAL: 5.2 | OTHER: 1.1 |

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The primary focus of work in the Molecular Genetics Section continues to be on control of cell proliferation in mammalian cells, with investigation of negative growth regulatory pathways emphasized within this framework. Several results of interest have been obtained. i) The short sequence motif GAGGCNGAGGC, which is partially responsible for growth regulatory properties of 7SL RNA and Alu elements, has been shown to bind a previously undiscovered protein containing tandem $C_2H_2$ class zinc fingers of the TFIIIA/*Krüppel* type. Characterized members of this family are transcription factors and master control genes in early embryogenesis. ii) A serious technical obstacle to use of RNA mobility shift assays in crude nuclear extracts has been overcome: high non-specific binding activity (which in the past completely masked specific RNA-protein interactions) was eliminated by high levels of the homopolymer poly(rG). This finding opens the way for the investigation of small RNA polymerase III-transcribed RNAs as nuclear regulatory factors. iii) Recombinant circle polymerase chain reaction (RCPCR), a novel method for site-specific mutagenesis and other molecular cloning procedures, was developed.

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